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THE UNIVERSITY OF ALBERTA

Characterization and Aquatic Toxicology of Heavy Oil

by

Detlef August Birkholz

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

PHARMACEUTICAL SCIENCES

(TOXICOLOGY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

FALL 1988

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Characterization and Aquatic Toxicology of Heavy Oil" submitted by Detlef August Birkholz in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Toxicology).

Supervisor

External examiner

Date: August 1, 1988

DEDICATION

This thesis is dedicated to my wife Karen, daughter Janelle and son Jared; their patience and understanding during the course of this research is appreciated.

ABSTRACT

Samples of diluted bitumen (bitumen and diluent naphtha, 1:1, v/v) and coker distillate (coker gas oil and coker naphtha, 2:1, v/v) were obtained from Syncrude Canada Ltd. and subjected to column fractionation followed by biological testing of the fractions using the *Salmonella*/microsomal assay (Ames Test) and the *Photobacterium phosphorium* assay (Microtox test). The most significant toxicity (as measured with the Microtox test) was ascribable to fractions containing polycyclic aromatic nitrogen heterocyclic compounds (PANH); particularly basic-PANH. Significant mutagenicity (as measured with the Ames test) was also observed for the basic PANH fraction. Analysis of this fraction by gas chromatography/high resolution mass spectrometry revealed the presence of alkyl-substituted polycyclic aromatic nitrogen heterocyclic compounds and alkyl-substituted quinolines.

An analytical method was developed for the determination of basic-PANH in fish tissue. This method was applied to study of the uptake, elimination, and biotransformation of 6,7-dimethylquinoline and 6,8-dimethylquinoline by rainbow trout (*Salmo gairdneri*). Both compounds were readily bioconcentrated by fish from water and eliminated following exposure and depuration. The major metabolites of 6,7-dimethylquinoline were observed to be conjugated (sulfate or glucuronide) alcohols, whereas the major metabolites of 6,8-dimethylquinoline were observed to be conjugated (sulfate or glucuronide) phenols and an alcohol. Concentrations of the metabolites in bile, after exposure to

approximately 1 mg/L of the dimethylquinolines in aquarium water, and 63 h of depuration with feeding, were observed to be 3 orders of magnitude above exposure levels.

Twenty-one alkyl-substituted quinolines were subjected to toxicity testing using the Microtox test. The observed toxicity (expressed as 5-min EC_{50}) varied over two orders of magnitude from 0.30 mg/L to 30 mg/L depending upon the degree and nature of substitution. Dimethylquinolines substituted in the 2 positions were observed to be less toxic than those isomers without a 2-substituent. Dimethylquinolines involving 5 or 6 substitution together with a 3-substituent were observed to be most toxic.

Three dimethylquinoline isomers were subjected to rainbow trout static fish bioassay. Results (expressed as 48h-LC_{50}) agreed with the observed 5-min EC_{50} determined with the Microtox test.

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Without the sincere effort of many people this research would not have been possible. I thank Dr. J. Aarts, Syncrude Canada Ltd., for providing the samples of coker distillate, coker naphtha, diluent naphtha and bitumen. Information on the operations of the Syncrude surface-mining plant, Ft. McMurray, Alberta was also appreciated. I wish to thank Environment Canada (Environmental Conservation & Protection, Western & Northern Region) for sponsoring this research. Special thanks are extended to Mrs. L. Scott (Environment Canada) for performing the Microtox tests; Mr. B. Bednar (Environment Canada) for performing the Ames tests and Mrs. L. Schwanbeck for her assistance with the chemical analysis.

I wish to thank Dr. W.L. Lockhart and Mr. R.W. Danell (Federal Department of Fisheries & Oceans, Winnipeg) for performing the static fish bioassays. Appreciation is also extended to Dr. S. E. Hrudey (University of Alberta, Dept. of Civil Engineering) for providing fish bioassay facilities and to Mr. R. Nelson for assisting in the fish exposure experiments.

Finally I wish to extend my sincere thanks to Dr. R.T. Coutts for his generous assistance, advice and patience during this lengthy research study.

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1. INTRODUCTION

The inevitable decline in global reserves of conventional crude oil has created an increased interest in heavy oil development. It has been estimated that 40% of the world's oil and 90% of Canada's oil supply is contained in heavy oil deposits (Hrudey and Scott, 1981). The majority of Canada's reserves are located in the oil sands deposits of Alberta. The Athabasca Oil Sands deposit in northeastern Alberta represents one of the world's most significant known hydrocarbon reserves. Current estimates suggest that synthetic crude oil reserves (recoverable by proven surface mining techniques) of 26.5 billion barrels are located there. This represents more than four times Alberta's current proven reserves of conventional crude oil (Hrudey *et al.*, 1976).

The hydrocarbon potential of this region is expected to result in rapidly accelerating industrial development. If this occurs, concerns will be raised over the environmental impact of such development especially during accidental release of raw materials and partially cracked raw materials to the environment as a result of spills, fires and equipment failures. A series of equipment failures did occur at an Alberta oil sands and bitumen upgrading plant and resulted in the release of more than 50 tons of oil (over several weeks) to the Athabasca River, under ice cover (Birkholz, *et al.*, 1987). During a subsequent investigation, it became evident that very little information existed on the types of chemical compounds present in heavy oil and partially upgraded heavy oil, and consequently, the environmental impact of such a spill was largely unknown. Initial

research revealed that the spilled material (heavy oil and partially upgraded heavy oil) contained numerous water-soluble components of environmental concern, including alkyl-substituted benzenes, polycyclic aromatic sulfur heterocycles, polycyclic aromatic nitrogen heterocycles and polycyclic aromatic hydrocarbons (Birkholz *et al.*, 1987). It also revealed that appropriate analytical methods for the isolation and identification of toxic chemicals in complex samples of heavy oil and partially upgraded (thermally cracked) heavy oil were not generally available. The development of such methods was perceived necessary in order to acquire meaningful information on the environmental impact of future spills. Furthermore, information obtained during the course of such method development could be useful to regulatory agencies for regulatory and monitoring purposes.

This research was relevant to the aims and objectives of Environment Canada, who supported the study financially. It is hoped that the information derived from this study can be used to assess and develop ways to reduce environmental threats from conventional/in-situ energy activities. The analytical methods developed during this research can provide regulatory agencies with the information required to monitor for toxic chemicals identified in this research in environmental samples during the course of future spills. Furthermore, the information obtained, from the toxicological assessments of heavy oil and its upgraded products, will be valuable in ranking the severity of environmental impact as a consequence of natural seepages of heavy oil into the environment versus accidental spills of partially ugraded heavy oil. The latter comparison of severity of

environmental impact is important because a common litigation defense argument has been that the environment surrounding heavy oil production is naturally polluted.

1.1 Process Description

Oil sands are mined in open pits and transported for extraction by conveyor systems. After rough screening to remove oversize material, the oil sand is mixed with hot water, steam and caustic in a conditioning drum (figure 1-1). From here the mixture is discharged to separation cells where gravity segregates the mixture into skimmable float, middlings and bottoms. The bottoms discharge to the tailings pond as wet tailings.

The middlings are transferred to a scavenger cell which uses air flotation to recover additional bitumen. This is combined with the skimmable float to constitute bitumen froth. The bitumen froth is diluted with raw diluent naphtha before centrifugation. The latter stage produces a bottoms stream which goes to tailings and the diluted bitumen which goes to storage prior to upgrading.

The diluent naphtha is recovered from the diluted bitumen by distillation immediately prior to charging the bitumen into the cokers. This latter process is a high temperature thermal cracking process which converts the predominantly high molecular weight bitumen molecules into smaller more useful hydrocarbons. The cokers at the Suncor plant in Alberta produce 5 streams: non-condensible gases, naphtha, kerosene, gas oil and solid coke. The middle three comprise the raw materials for blending to produce synthetic crude. The cokers

at the Syncrude plant in Alberta produce 4 streams: non-condensible gases, naphtha, gas oil and solid coke. Coker naphtha and gas oil comprise the raw materials for blending to produce synthetic crude. These blended streams (blended to produce synthetic crude) contain substantial quantities of unsaturated hydrocarbons and sulfur and nitrogen heterocycles, directly from the coker. These materials are undesirable in petroleum products so the coker distillate (refers to coker naphtha and gas oil at the Syncrude plant, and coker naphtha, kerosene and gas oil at the Suncor plant) is hydrogen treated in separate hydrotreating stages to saturate the unsaturated hydrocarbons and remove sulfur and nitrogen compounds. The hydrotreated product is blended to produce synthetic crude.

1.2 Study Design

From figure 1-1 (and discussions with Syncrude Canada) it became apparent that the major streams present in a heavy-oil mining and upgrading plant are diluted bitumen (bitumen plus diluent naphtha) and coker distillate (coker gas oil and coker naphtha, Syncrude plant). Because of the large size of these streams relative to other process streams, any plant upset would likely result in the release of greater proportions of these streams to the environment than other process streams. Therefore, attention was focused on studies of these two streams.

Accordingly samples of bitumen, diluent naphtha, coker naphtha and coker gas oil were obtained from Syncrude Canada Ltd. and combined to form two samples namely diluted bitumen (diluent naphtha and

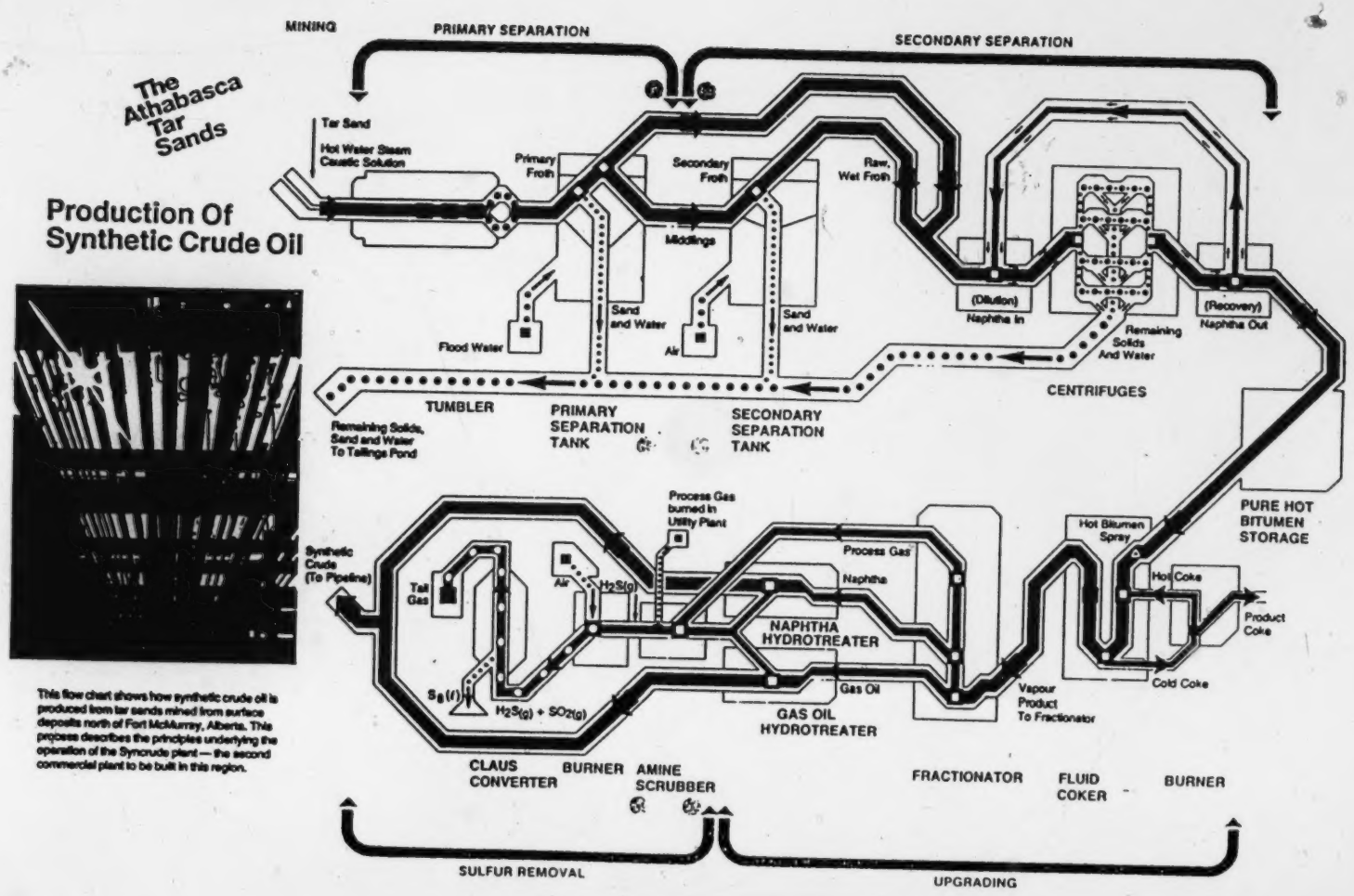

Figure 1-1 FLOW CHART SHOWING HOW SYNTHETIC CRUDE OIL IS PRODUCED BY A SURFACE-MINING PLANT (OBTAINED FROM SYNCRUDE CANADA LTD.)

bitumen, 1:1, v/v) and coker distillate (coker naphtha and coker gas oil, 1:2, v/v). These two samples were subjected to exhaustive column fractionation and analysis followed by bioassay in order to meet the objectives of this study.

1.3 Objectives

1. Development of an analytical protocol for the isolation and characterization of toxic chemicals in complex heavy oil process samples. Included in this objective are the standardization of separation procedures (such as chemical class separation) and repeated confirmation of the performance of developed procedures by testing model compounds and analyzing complex heavy oil samples.

2. Isolation and identification of the most toxic components present in diluted-bitumen and coker-distillate.

3. Determination of the uptake, elimination, and biotransformation of a representative number of toxic chemicals by fish.

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2. AQUATIC TOXICOLOGY OF HEAVY OIL

2.1. Introduction

The inevitable decline in global reserves of conventional crude oil has created increasing interest in heavy oil development. It has been estimated that 40% of the world's oil and 90% of Canada's oil supply is contained in heavy oil deposits (Hrudey and Scott, 1981). The emergence of developing technologies to meet world energy needs (such as heavy oil development) imposes new requirements on existing environmental analytical capabilities. These emerging technologies present unknown challenges to the environment and it is important to develop analytical schemes capable of comprehensively characterizing potential environmental problems.

Such a challenge came to light in 1982 when a fire and a series of equipment failures at the Suncor oil sands plant, north of Ft. McMurray, Alberta, resulted in a spill of coker distillate fractions, including raw naphtha, to the plant's wastewater pond. Over a subsequent 10 week period, plant monitoring records reported the release of more than 50 tons of oil and grease to the Athabasca River under winter ice cover (Birkholz, *et al.*, 1987). Although numerous investigations into the composition of bitumen and heavy oil have been performed (Bunger *et al.*, 1979; Selucky *et al.*, 1977; Strausz, 1984), little research has been conducted on the composition of thermally cracked, or upgraded bitumen. Consequently, the environmental impact

of spills of bitumen and partially cracked bitumen to the environment are difficult to assess. Compounds of environmental concern such as alkyl-substituted benzenes, polycyclic aromatic hydrocarbons (PAH), benzothiophenes, and dibenzothiophenes have been identified in coker naphtha, coker gas-oil, and middle distillate fractions of heavy oil (Cooley *et al.*, 1981; Poirier and Smiley, 1984). The environmental toxicology of these compounds has been reviewed by Vandermeulen (1987) and discussed by Birkholz *et al.*(1987). Water-soluble compounds were isolated from spilled coker distillate fractions and identified as saturates, acridine, cyclic ketones, alkyl-substituted benzenes, benzothiophenes, furans, indans, quinolines, and dibenzothiophenes. From these studies it is clear that many classes of organic compounds are present in coker distillate derived from thermally cracked bitumen. This is not surprising in view of the known (Selucky *et al.*, 1977) composition of bitumen: 83.15% C, 10.3% H, 0.40% N, 1.6% O and 4.58% S. Thermal cracking of such a complex substance would certainly result in the formation of many classes of organic compounds.

To better understand the environmental impact of future spills of coker distillate and diluted bitumen fractions, samples of coker distillate (coker naphtha + coker gas-oil, 1:2, v/v) and diluted bitumen (bitumen + diluent naphtha, 1:1, v/v) were obtained from a surface mining plant and subjected to chemical fractionation using a modified version of the method of Later *et al.*, (1981). The resulting fractions were then subjected to toxicity testing using the *Salmonella*/microsomal assay (Ames test) and the *Photobacterium phosphorium* assay (Microtox test). All fractions were subjected to

chemical analysis using screening techniques such as gas chromatography/flame ionization detection (GC/FID), gas chromatography/flame photometric detection (GC/FPD), gas chromatography/nitrogen phosphorous detection (GC/NPD), high performance liquid chromatography/ultraviolet detection (HPLC/UV) and high performance liquid chromatography/fluorescence detection (HPLC/FLUOR). Toxic fractions derived from coker-distillate were subjected to analysis by gas chromatography/low resolution mass spectrometry (GC/LRMS) and gas chromatography/high resolution mass spectrometry (GC/HRMS). The results of this study are now presented.

2.2. Experimental Section

2.2.1. Heavy Oil Samples

Samples of diluent naphtha, bitumen, coker gas-oil, and coker naphtha were provided by Syncrude Canada Ltd. and were representative of products derived from that company's surface mining plant in Fort McMurray, Alberta. A diluted bitumen sample was prepared following Company instructions by diluting bitumen with equal volumes of diluent naphtha. A coker-distillate sample was similarly prepared by mixing two volumes of coker gas-oil with one volume of coker naphtha. Coker-distillate and diluted-bitumen represent the major products (volume basis) processed by the Syncrude plant. In the case of a major plant upset, they would have greatest potential for discharge to the environment.

2.2.2. Column Fractionation

Three hundred milligram aliquots of diluted-bitumen and coker-distillate were subjected to alumina chromatography using a modification of the method of Later *et al.*, (1981). Briefly, neutral alumina (100 mesh, Camag) was placed in a Soxhlet extractor and extracted overnight with dichloromethane. Following removal of the solvent by heating for several hours in a convection oven maintained at 130°C, the adsorbent was cooled in a desiccator and 10 g aliquots were weighed into glass scintillation vials. The vials were placed into a muffle furnace and the adsorbent activated at 400°C overnight. After cooling the adsorbent in a desiccator for 2-3 h, the vials were capped and sealed with teflon tape. Prior to chromatography, the adsorbent was deactivated with various quantities of organic-free water, ranging from 1.0 to 1.5% (w/w) and standardized by chromatographing a series of model compounds following the procedure of Later *et al.*, (1985). The model compounds used were n-pentacosane, phenanthrene, benzo(g,h,i)perylene, 2-methylindole, benzo(h)quinoline, 3-aminofluoranthene, and 2-naphthol. Later *et al.*, (1985) recommended the use of dibenzo(def,mno)chrysene during the standardization process. A sample of this substance was not available to us, so it was replaced with a similar compound benzo(g,h,i)perylene. This standardization process indicated that best separation was achieved on alumina deactivated with 1.5% water so we used similarly deactivated alumina. Samples of coker-distillate (300 mg) were separately dissolved in a few mL of chloroform and applied to 7 columns of 3 g of

deactivated alumina. After removal of the solvent by means of a stream of nitrogen, the alumina, with adsorbed sample, was applied to a 1 cm i.d. chromatography column containing 6 g of deactivated alumina. Fractionation was performed by succesive elution with hexane (20 mL, fraction A1), benzene (50 mL, fraction A2), chloroform (100 mL; containing 0.75% ethanol as preservative, fraction A3) and 1% acetic acid in methanol (200 mL, fraction A4).

The chloroform fraction (A3), obtained from the chromatography of coker-distillate on alumina, was further fractionated by performing an acid/base partition. Briefly, 3 mL of the combined fraction was diluted to 25 mL with chloroform and serially extracted with 3 x 25 mL of 6N hydrochloric acid (HCl). The upper HCl layer was removed, collected in a 250 mL Erlenmeyer flask, cooled in an ice bath, and basified using 6 N sodium hydroxide (NaOH) to pH > 10. Following extraction of the basified solution with 3 x 25 mL of chloroform, the extract was dried by passage through a 20 g sodium sulfate column, and concentrated to 2 mL with the aid of a rotary evaporator and a nitrogen evaporator. This extract (A3-base) contained strong bases. The chloroform extract, containing acid/neutral material, was similarly dried through a 20 g sodium sulfate column and concentrated to 2 mL using a combination of rotary evaporation and nitrogen blowdown to give extract A3-acid.

The diluted-bitumen sample (300 mg) was subjected to an acid/base partition prior to chromatography by dissolving 300 mg of sample in 5 mL of chloroform and serially extracting with 3 x 5 mL of alkaline water (pH > 11). The chloroform layer was dried by passage through a

sodium sulfate drying column and preconcentrated prior to alumina chromatography (fraction, DB-B/N). The aqueous phase (15 mL) was acidified to pH < 2 by the addition of 6 N sulfuric acid and serially extracted with 3 x 5 mL of chloroform. After drying the chloroform extract by passage through a sodium sulfate column, both the chloroform (fraction, DB-acid) and remaining water (fraction, DB-aqueous) were preconcentrated to 5 mL with the aid of a rotary evaporator. Seven aliquots of coker-distillate (300 mg per aliquot) were chromatographed on alumina and the four fractions (A1-A4) obtained were pooled and preconcentrated to 5 mL. Similarly, five aliquots of diluted-bitumen were subjected to acid/base partition followed by chromatography on alumina of the base/neutral portion (DB-B/N). All chromatographic fractions (A1-A4) as well as the acid extract (DB-acid) and the aqueous phase (DB-aqueous) were pooled and concentrated to 5 mL.

2.2.3. Acetylation of Fraction A4

The methanol: acetic acid eluant (99:1), obtained from the chromatography of coker-distillate on alumina (fraction A4), was acetylated following the procedure of Hargesheimer *et al.* (1981). One mL of the fraction (in methanol: acetic acid, 99:1) was transferred to a 50 mL centrifuge tube and 4 ml of water along with 250 μL of acetic anhydride was added. After vortexing the mixture for 1 min, solid sodium bicarbonate (Fisher Scientific) was added and mixing continued until the evolution of CO_2 ceased and an excess of sodium bicarbonate was apparent. The aqueous solution was then extracted with 3 x 1 mL of

chloroform, the combined extract was dried by passage through a 1 g sodium sulfate column and collected in a 5 mL calibrated centrifuge tube. Volume was adjusted to 1.0 mL with the aid of a nitrogen evaporator (fraction A4-acetylated).

2.2.4. *Salmonella*/microsomal assay (Ames test)

The Ames test was conducted on all the fractions obtained as described above, using the procedure of Maron and Ames (1983). Aliquots of fractions (200 - 400 μL), obtained from the chromatography of diluted-bitumen and coker-distillate (A1-A4), as well as the acid fraction of diluted-bitumen (DB-acid) and the acid (A3-acid) and base fraction (A3-base) obtained from the chloroform fraction (A3) of coker-distillate, were transferred to a 1 mL tared vial and the solvent was removed with the aid of nitrogen. The resulting residue was weighed and reconstituted with 400 μL of 1,4-dioxane prior to assay. In addition, aliquots (10, 20, 35, and 50 μL) of coker-distillate, and diluted-bitumen fractions were assayed using tester strain TA 98 with and without the addition of microsomal enzymes (Aroclor 1254 induced rat liver homogenate, 9000-g fraction). The aqueous fraction (DB-aqueous) obtained from diluted-bitumen was analyzed directly by assaying aliquots of the same size described for the chromatographic fractions. Quality assurance was maintained by following the recommendations of Williams, (1985). For example, spontaneous reversion count tests were performed in triplicate and standard mutagens (positive controls) were assayed in duplicate. Method blanks were also assayed for fractions showing a mutagenic

response. A minimum of two plates per dose were prepared as recommended (Williams, 1985). The standard mutagens employed were daunomycin and benzo(a)pyrene. Solutions of both mutagens were prepared in water and 1,4-dioxane respectively. Data reduction followed the general procedures given by DeSerres and Shelby (1979) and Williams (1985).

2.2.5. *Photobacterium phosphorium* assay (Microtox test)

Bacterial toxicity assays were performed using a Microtox toxicity analyzer. The analyzer, lyophilized luminescent bacteria (*Photobacterium phosphoreum*), and other assay reagents were supplied by Microbics Operations of Beckman Instruments, Inc., Carlsbad, California. The instrument was equipped with a photomultiplier tube located near a reaction chamber which had an adjustable temperature range of 10 to 25°C. The analyzer was also equipped with a ten-well incubator chamber which held the test temperature at 15 ± 0.3°C in all assays. Light output was monitored using a chart recorder.

All Microtox bioassays were performed in duplicate using previously described procedures (Bulich *et al.*, 1981 and Lebsack *et al.*, 1981), and are summarized as follows. Aliquots (200 - 500 μL) of fractions obtained from the chromatography of diluted-bitumen and coker-distillate (A1-A4), as well as the acid fraction of diluted-bitumen (DB-acid) and the acid (A3-acid) and base fraction (A3-base) obtained from the chloroform fraction (A3) of coker-distillate, were transferred to a tared 1.5 mL ampoule. Following the removal of the solvent with the aid of nitrogen, each residue was weighed and

dissolved in 50 - 100 μL of tetrahydrofuran (THF). The solution was then quantitatively transferred to a 200 or 250 mL volumetric flask by rinsing the ampoule with an additional 50 μL of THF and diluting to volume with distilled and deionized water. Method blanks for all fractions were evaluated in the same fashion as the sample fractions. In another set of experiments tared residues obtained from the fractionation of coker-distillate and diluted-bitumen (A1-A4, DB-acid, DB-aqueous) were diluted to 10 mL with distilled water and subjected to Microtox testing. Aliquots (1.8 mL) of the aqueous solutions, prepared from the fractions obtained from the chromatography of coker-distillate and diluted-bitumen (A1-A4), as well as the aqueous fraction derived from diluted-bitumen (DB-aqueous) and the acid (A3-acid) and base fraction (A3-base) derived from the chloroform fraction (A3) of coker-distillate, were mixed with 0.2 mL of 20 percent sodium chloride solution to yield an initial concentration of 90 percent. All subsequent sample dilutions were made from this solution, using the Microtox diluent, so that 45, 22.5, 11.25, and 5.63 percent of the original concentration were tested.

The Microtox reagent (lyophilized bacteria) was reconstituted with Microtox reconstitution solution, and 0.01-mL aliquots were transferred to cuvettes containing 0.5 mL Microtox diluent equilibrated (about 10 min) at 15°C. Initial light measurements were made three times for each cuvette containing bacterial cell suspension. Diluent control and sample dilutions, equilibrated to 15°C in the incubator wells, were added (0.5 mL) from the corresponding cuvettes to the luminescent bacterial suspensions. Light measurements

were then made by cycling cuvettes through the turret, at 1-min intervals, for 5 to 15 min to obtain the final light output readings for each cuvette. The diluent control (blank) was used to correct time-dependent drift in sample light output. The effective concentration producing 50% light reduction (EC_{50}) for all bioassays was calculated using the initial, 5 min and 15 min light levels following the described method of the manufacturer and Bulich (1982). Data reduction employed the gamma function (Γ) which is the ratio of the amount of light lost to the amount of light remaining. Details of this data reduction process are described by Bulich (1982). The EC_{50} values are expressed in milligrams per litre (mg/L) for all fractions tested.

2.2.6. Gas Chromatography

Gas chromatography/flame ionization analysis (GC/FID), was performed using a Hewlett-Packard (HP, model 5880) gas chromatograph. Following the automatic splitless injection of 2 μL of sample onto a 30 m x 0.25 mm i.d. fused silica capillary column (wall-coated with DB-5, 0.25 μm film thickness, J&W Scientific), the oven temperature was increased from 40 to 300^{o}C at 2^{o}C/min beginning 1 min after injection. The oven temperature was maintained at 300^{o}C for 5 min and the injector and detector were maintained at 270 and 300^{o}C respectively throughout the entire chromatographic procedure. The carrier gas was helium (linear velocity, 25 cm/sec at 300^{o}C) and the injector purge valve was opened 30 sec after injection.

Gas chromatography/flame photometric analysis (GC/FPD), was

performed using a Tracor model 560 gas chromatograph. After the splitless injection of 2 μL of sample onto a 25 m x 0.32 mm i.d. fused silica capillary column (wall-coated with SE-54, 0.17 μm film thickness, HP), the oven temperature was increased from 50 to 250°C at 10°C/min beginning 1 min after injection. The oven temperature was maintained at 250 °C for 5 min and the injector and detector were maintained at 250°C throughout the procedure. The carrier gas was helium and the linear velocity was 31 cm/sec at 250°C. The injector purge valve was opened 40 sec after injection.

Gas chromatography/nitrogen phosphorus detection (GC/NPD) was performed using a HP model 5880 GC. Following the splitless injection of 2 μL of sample onto a 30 m x 0.25 mm i.d. fused silica capillary column (wall-coated with DB-5, 0.25 μm film thickness, J&W Scientific) the oven temperature was increased from 40 to 300°C at 10°C/min beginning 1 min after injection. The injector and detector temperatures were maintained at 270 and 300°C respectively throughout the procedure. The carrier gas was helium, which maintained a linear velocity of 25 cm/sec at 300°C, and the injector purge valve was closed 30 s after injection.

2.2.7. Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (low resolution), was performed by interfacing a HP model 5890A GC to a HP model 5970 quadrupole mass spectrometer. Injection of 2 μL of sample (via a splitless injector) was made onto a 12.5 m x 0.2 mm i.d. fused silica, wall-coated HP-1 capillary column obtained from HP. The carrier gas

was helium (linear velocity was 35.5 cm/sec at 300°C), and the temperature was increased from 40 to 300°C at 10°C/min beginning 1 min after injection. The oven temperature was maintained at 300°C for 8 min, and the injector, transfer line and ion source were maintained at 250, 300 and 220°C respectively throughout the entire procedure. Data was acquired, beginning 2 min after injection using a HP model 59970C data system. Mass spectral scans (from 35 to 350 amu) were obtained every 1.36 seconds.

Gas chromatography/high resolution mass spectrometry was performed by interfacing a Varian model 6000 GC with a Vacuum Generators model 70E magnetic sector mass spectrometer. After the splitless injection of 2 μL of sample onto a 60 m x 0.25 mm i.d. DB-5 fused silica capillary column (film thickness 0.25 μm, J&W Scientific), the oven temperature was programmed from 50 to 300°C at 3°C/min and held at this temperature for 5 min. The injector and source temperature were maintained, throughout the procedure, at 310 and 260°C respectively. Mass spectral scans were made over the range of 50 - 600 amu at a rate of 1 sec/decade. Mass resolution was adjusted to 5000. The carrier gas was helium, which maintained a linear velocity of 35 cm/sec at 250°C, and the injector purge valve was opened 30 sec after injection.

2.2.8. High Performance Liquid Chromatography

Analyses using high performance liquid chromatography (HPLC) were conducted with a Waters (Millipore Corp., Milford, MA) high pressure liquid chromatograph. The system consisted of two M 6000 pumps, a

model 680 solvent programmer, a model 710B autosampler, a model 450 variable wavelength detector and a model 420-AC fluorescence detector. The variable wavelength detector was set at 0.1 AUFS and the wavelength was 254 nm. The fluorescence detector employed a 254 nm band pass excitation filter and a 365 nm long pass emission filter. Attenuation was set to 8x. The detectors were configured in series and the output was monitored with a Fisher Recordal, model 5000 dual channel 10 mv strip chart recorder. The column employed was a 4.6 mm i.d. x 150 mm Vydac 201TP Reverse Phase with 5 μm column packing (Technical Marketing).

After the injection of 5 μL of sample (or dilutions thereof), the solvent strength was increased linearly from 50% acetonitrile in water to 100% acetonitrile, over a 15 min time period. The gradient was maintained at 100% acetonitrile for 5 min before returning to intial conditions. The flow rate was maintained at 3 mL/min.

2.2.9. Infrared Analysis

The acid fraction derived from diluted-bitumen (DB-acid) was subjected to an infrared (IR) scan using a Perkin-Elmer, model 457, grating, infrared spectrophotometer. The sample (175 mg/200 μL of chloroform) was placed in a 0.25 mm NaCl cell and scanning intiated from 4000 to 800 cm^{-1}. The scan speed was medium and the slit set at normal. The spectrum was recorded on IR chart paper.

2.2.10. Materials

Normal paraffins (C_7 - C_{40}) were obtained neat from Supelco. A

solution containing these paraffins was prepared in dichloromethane and used to monitor for the presence of these compounds in samples.

A similar kit containing alkyl-substituted phenols was also obtained from Supelco and solutions containing these substances were prepared in dichloromethane.

A solution containing known concentrations of priority pollutant phenols was obtained from the United States Environmental Protection Agency Quality Assurance Repository in Cincinnati, Ohio. This solution was acetylated as described in section 2.2.3. and analyzed by GC/MS.

Polycyclic aromatic hydrocarbons (PAH) were obtained from the United States Environmental Protection Agency, Quality Assurance Repository in Cincinatti, Ohio. Substituted naphthalenes were obtained from Aldrich and reported to be greater than 99% pure. A solution containing PAH in dichloromethane was prepared and used to monitor for their presence in samples.

Fourteen isomers of dimethylquinoline (table 2-13), as well as 3-ethylquinoline were synthesized in the University of Alberta Chemistry Department using the procedure of Manske *et al.*, (1942). Purity of the compounds was determined by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS) and found to be greater than 97% for all compounds except 7,8-dimethylquinoline and 3,8-dimethylquinoline which were observed to be 69 and 94% pure respectively.

Solvents were distilled in glass (Fisher); tetrahydrofuran had to be fractionally distilled prior to use.

2.3. Results and Discussion

2.3.1. Chromatography of Model Compounds and Samples

Chromatographic separation of model compounds on 1.5% (w/w) deactivated alumina is illustrated (table 2-1). From this table it is apparent that saturated hydrocarbons (e.g. n-pentacosane) are confined exclusively to fraction A1; polycyclic aromatic hydrocarbons (PAH) (e.g. phenanthrene and benzo(g,h,i)perylene are confined exclusively to fraction A2; polycyclic aromatic nitrogen heterocycles (PANH) (e.g. 2-methylindole, benzo(h)quinoline, and 3-aminofluoranthene) are confined exclusively to fraction A3; and hydroxy polycyclic aromatic hydrocarbons (HPAH) (e.g. 2-naphthol) are confined exclusively to fraction A4. This selectivity is consistent with the proper deactivation of this adsorbent (Later *et al.*, 1985) and the excellent recoveries of the model compounds provided further confidence in this separation method.

The calculated mass balances for the various chromatographic fractions obtained from diluted-bitumen (A1-A4) are listed in table 2-2. Total average recovery of the base/neutral component was 63%. This low recovery was attributed to the loss of volatile diluent naphtha during the solvent evaporation step. This was later confirmed by weighing an aliquout of diluent-naphtha and gently concentrating it to constant weight with nitrogen. Thirty to forty percent losses in weight were observed by this process.

The calculated mass balances obtained during the fractionation of coker-distillate are given in table 2-3. A mean recovery of 106% (w/w) for the fractionated sample was observed. It is also of interest to note that fraction A1, which contains saturates, comprises 38% (w/w) of the sample, and the PAH and PASH fraction (A2) comprises 44% (w/w) of the sample. Fraction A3, which contains PANH, comprises 6.4% (w/w) of the sample. This fraction contains 98% (w/w) acid/neutral or weakly basic material and 2% (w/w) strongly basic material.

In order to verify the chemical class separation of complex samples such as diluted-bitumen and coker-distillate, fractions (A1-A4) obtained from these samples were subjected to analysis using GC/FID, GC/FPD, GC/NPD, GC/MS, HPLC/UV and HPLC/FLUOR. Figures 2-1 to 2-3 are the GC/FID chromatograms obtained for fractions A1-A3 derived from diluted-bitumen. Other than those from fraction A1 (figure 2-1) very few prominent chromatographic peaks are evident. GC peaks associated with the presence of saturates (via GC retention time) are shown in figure 2-1. Very few saturates are observed to be present. GC peaks associated with the presence of specific PAH are shown in figure 2-2. Very small peaks associated with these compounds were observed.

Figures 2-4 to 2-6 are GC/FID chromatograms for fractions A1-A3 derived from coker-distillate. The presence of many GC peaks in all fractions is apparent. Fraction A1 (figure 2-4) is dominated by saturate material as evidenced by the presence of GC peaks with similar retention times to authentic n-paraffins. Saturates from C_7 to C_{24} are evident. Fraction A2 (figure 2-5) contains mainly PAH material

as evidenced by the presence of GC peaks with retention times similar to those of authentic PAH. Fraction A3 (figure 2-6) also contains many GC peaks, in contrast to the very few peaks observed in the equivalent fraction A3 obtained from diluted-bitumen (figure 2-3).

Figures 2-7 to 2-9 are GC/FPD chromatograms of fractions A1-A3 derived from diluted-bitumen. Only fraction A1 (figure 2-7) is rich in components.

Figures 2-10 to 2-12 are GC/FPD chromatograms of fractions A1-A3 derived from coker-distillate. A complex mixture of sulfur- containing compounds is apparent in fraction A1 (figure 2-10). This chromatogram clearly represents low molecular weight sulfur containing compounds; the chromatogram is devoid of GC peaks after 13 min. The chromatogram of fraction A2 (figure 2-11) was complex. This fraction contained high molecular weight material as evidenced by the presence of GC peaks with long retention times. Fraction A3 (figure 2-12) contained very few GC peaks.

GC/NPD chromatograms of fractions A1-A3, derived from diluted-bitumen, and fractions A1-A2, derived from coker-distillate are provided in figure 2-13. Very few GC peaks are apparent. Figure 2-14 is a GC/NPD chromatogram of fraction A3, derived from coker-distillate. A complex chromatogram, similar in profile to the GC/FID chromatogram obtained for this fraction (figure 2-6) is apparent.

The HPLC/UV and HPLC/FLUOR chromatograms for fractions A1-A4 obtained for diluted-bitumen are provided (figures 2-15 to 2-18). Fluorescent and UV-absorbing material is evident primarily in fraction A2 (figure 2-16) and fraction A3 (figure 2-17); a minimum of 19 μg of

material had to be injected on column in order to elicit a fluorescent response.

Figures 2-19 to 2-22 are HPLC/UV and HPLC/FLUOR chromatograms of fractions A1-A4 obtained for coker-distillate. Fluorescent responses (20-30 % full scale deflection, FSD) were observed primarily in fraction A2 (figure 2-20) and fraction A3 (figure 2-21) after the injection of 2.5 to 3.3 μg of sample. A fluorescence response (40% FSD) was also observed for fraction A4; however 88 μg of sample had to be injected to elicit this response.

The following conclusions can be reached after review of the information contained in figures 2-1 to 2-22.

1. Diluted-bitumen contains very little gas-chromatographable material, indicative of low levels of saturates, PAH, PASH, PANH and HPAH. This conclusion arises from the observed poor GC/FID, GC/FPD, GC/NPD, HPLC/UV and HPLC/FLUOR responses. GC/FID analysis did reveal the likely presence of saturate material (C_7 - C_{10}) in fraction A1 (figure 2-1) and this finding is consistent with the low boiling range of diluent naphtha which comprises 50% (w/w) of this sample. Chemical characteristics of diluent naphtha are described in table 2-4. The volatility of diluent-naphtha is also responsible for the poor mass balance obtained for the chromatographic fractions (A1-A4). Although a GC/FPD response was obtained for fraction A1 (figure 2-7), the absence of a strong fluorescence response for this fraction (figure 2-15) discounts the presence of PASH. The presence of sulfur-containing compounds is expected since diluent naphtha contains 10 μg/g sulfur (table 2-4). The absence of a GC/NPD response suggests very little

PANH to be present in diluted-bitumen and the very weak fluorescent response for fraction A4 (figure 2-18) suggests very little HPAH material is present.

In stark contrast, analysis of coker-distillate by GC/FID, GC/FPD, GC/NPD, HPLC/UV and HPLC/FLUOR revealed the presence of many classes of compounds. Saturates are present in fraction A1 (figure 2-4) because the GC retention times of select peaks agreed with those of authentic n-paraffins. The presence of PAH and PASH in fraction A2 was intially surmised from the GC/FID (figure 2-5), GC/FPD (figure 2-11) and HPLC/FLUOR (figure 2-20) responses for this fraction, and confirmed by direct comparisons of chromatographic retention times with those of authentic PAH, and by correlating retention times with those of components in fraction A2 (figure 2-5). The presence of volatile sulfur containing compounds in fraction A1 (figure 2-10) is not unexpected. Coker-naphtha comprises 33.3% (w/w) of the sample and contains 1.79% (w/w) sulfur (table 2-5). The presence of PANH in fraction A3 is suggested from the GC/FID (figure 2-6), GC/NPD (figure 2-14) and HPLC/FLUOR (figure 2-21) responses for this fraction. The similar profile obtained for both the GC/FID (figure 2-6) and GC/NPD (figure 2-14) chromatograms suggests a nitrogen-enriched fraction. The positive fluorescence response for fraction A4 (figure 2-22) suggests the presence of HPAH. This was confirmed by reanalysis of fraction A4 after acetylation (fraction A4-acetylated) using GC/MS. This analysis revealed the presence of substituted phenols and naphthols. Structural identifications of phenols and naphthols were made by direct comparisons of their mass spectra with those of authentic reference

compounds. A total ion chromatogram for fraction A4-acetylated (derived from coker distillate) is shown in figure 2-23. A summary of the mass spectra obtained for fraction A4 is provided in Table 2-6.

The complexity of coker-distillate is not unexpected since a review of the chemical properties of coker-naphtha (table 2-5) and coker-gas-oil (table 2-7) reveals high levels of S and N and simulated distillation curves suggest that most of the material is sufficiently volatile for gas chromatographic analysis. On the other hand, a review of the chemical properties of diluent-naphtha (table 2-4) and bitumen (table 2-8) also reveals high levels of S and N. In this instance, the simulated distillation curves indicate that the naphtha component is likely the most volatlile, and that the bitumen portion is the least volatile.

Figure 2-24 is the IR spectrum obtained from diluted-bitumen and represents the acid fraction (DB-acid). This spectrum is similar to that obtained by Zenon (1986) and MacKinnon and Boerger (1986) and has been attributed to the presence of naphthenic acids.

2.3.2. Microtox Assay

A summary of the Microtox results is presented in table 2-9. From this table it is apparent that the most toxic fractions are A3 and A4 derived from coker-distillate and fractions A4 and acids (DB-acid) derived from diluted-bitumen. It is interesting to note that the 5-min EC_{50} values obtained for aqueous solutions of coker-distillate and diluted-bitumen differ significantly from 5-min EC_{50} values obtained using a solvent carrier (1,4-dioxane). Furthermore,

fractionation of A3 derived from coker-distillate into acid/neutral (A3-acid) and strongly basic (A3-basic) components revealed an enhanced toxicity of the basic component. The toxicity associated with the acid fraction of diluted-bitumen (DB-acid) is not surprising since similar findings have been reported by MacKinnon and Boerger (1986). These researchers identified the toxic acidic compounds to be naphthenic acids. The presence of naphthenic acids in this fraction was confirmed by comparing an IR spectrum obtained for this fraction to that published by Zenon (1986) and MacKinnon and Boerger (1986). The observed toxic response for coker-distillate fraction A4 is also not unexpected since the presence of phenols is known to elicit a toxic response with the Microtox test (Lebsack *et al.*, 1981). The presence of phenols was confirmed in fraction A4 derived from coker-distillate by GC/MS analysis (table 2-6).

Quality control on all Microtox tests was maintained by replicate testing of an aqueous solution of hexachloroethane. The mean 5-min EC_{50} was determined to be 0.31 mg/L (n = 16). This value agrees reasonably well with the value of 0.14 mg/L reported by Curtis *et al.* (1982).

2.3.3. *Salmonella*/Microsomal Assay (Ames Test)

Genotoxicity results obtained with the Ames test are summarized in table 2-10. Mutagenicity was observed for fractions A3 and A4 derived from coker distillate. A linear dose response (r^2 = 0.83-0.86) was observed for replicate assays of fraction A3 and a linear dose response (r^2 = 0.86 - 0.96) was observed for replicate assays of

fraction A4. These regressions were obtained by plotting the observed revertants against three different extract doses and the control. Although four extract doses were intially applied, toxicity was observed at the highest dose and therefore data for this dose was not included in the analysis. Response for the lowest dose was 2 - 12 fold higher that the spontaneous revertant count. Direct-acting mutation was observed for both of these fractions. Using linear regression analysis, 10 mg of fraction A3 extrapolated to 464 - 776 revertants, and 1 mg of fraction A4 extrapolated to 150 revertants. The mean spontaneous reversion count (n = 6) was 16 revertants per plate.

Fractionation of coker-distillate A3 into acid/neutral (A3-acid) and basic material (A3-basic) followed by retesting revealed a linear dose response for A3-acid (r^2 = 0.96) and a similar response for A3-basic (r^2 = 0.98). The linear regression analysis for the acid/neutral (A3-acid) samples was obtained by plotting the observed revertants against four different extract doses and the control in duplicate. The same analysis for the basic fraction (A3-basic) was obtained by plotting observed revertants against three extract doses and the control. Initially four extract doses for A3-basic were applied but, the largest dose was toxic and the data for this dose was not included in the analysis. The number of revertants observed for the lowest dose was 3 - 10 fold higher than the spontaneous revertant count. Using linear regression analysis, 10 mg of fraction A3-acid was extrapolated to 670 revertants, whereas 1 mg of fraction A3-basic was extrapolated to 2390 revertants. The acid/neutral (A3-acid) material was observed to be direct acting whereas the basic

material (A3-basic) required liver activation.

The results summarized in tables 2-9 and 2-10 clearly show that fraction A3 derived from coker-distillate is both acutely lethal and genotoxic. Furthermore, basic components (A3-basic) isolated from this fraction are significantly more lethal and genotoxic than the original fraction (A3). In the interests of identifying the components responsible for this observed toxicity, the basic fraction (A3-basic) was subjected to analysis by high resolution GC/MS.

Quality control for the Ames test was maintained by including standard mutagens, namely daunomycin and benzo(a)pyrene in the assay procedure. Using linear regression analysis, 1.0 μg of benzo(a)pyrene extrapolated to 171 - 190 revertants. This response was consistent with that reported by Maron and Ames (1983) who showed that 1.0 μg produced 143 revertants. Our observed response for daunomycin was significantly higher than that reported by Maron and Ames (1983). Six μg of daunomycin produced over 6000 revertants in our test whereas Maron and Ames (1983) reported just over 3000. Although our spontaneous revertant count was low (mean = 16 without liver homogenate (S9), n = 6) relative to that suggested by Maron and Ames (1983) (mean = 30 - 50 without S9), our responses for standard mutagens were generally higher than those reported by Maron and Ames (1983). Therefore, the test was judged to be providing meaningful data. Linear dose responses were observed for daunomycin and benzo(a)pyrene with correlation coefficients (r^2) of 0.98 and 0.94, respectively.

2.3.4. Gas Chromatography/Mass Spectrometry Analysis

Figure 2-25 is a total ion chromatogram obtained for the basic fraction obtained from coker-distillate (A3-basic). A complex chromatogram containing many GC peaks is apparent. High resolution mass spectra were retrieved and mass assignments made. This information is summarized in table 2-11. Compounds identified in this fraction include substituted quinolines and nitrogen-containing polycyclic aromatic hydrocarbons (PANH). Proposed chemical structures for the PANH are shown in figure 2-26. It is recognized that many chemical isomers are possible and that the position of the nitrogen heteroatom is not necessarily correct. Reanalysis of this fraction by GC/low resolution mass spectrometry generated the total ion chromatogram shown in figure 2-27. Ion histograms characteristic of the compounds identified by GC/high resolution mass spectrometry are shown in figures 2-28 and 2-29 and confirm the presence of substituted quinolines and substituted PANH. Figure 2-30 is a total ion chromatogram obtained for the basic compounds present in coker-distillate fraction A3 (A3-basic). The total ion chromatogram focuses on the retention time window 9 - 13.5 min. A complex chromatogram is apparent as evidenced by the presence of many GC peaks. A selected ion chromatogram for m/z 157 (indicative of dimethyl- or ethylquinoline) over the retention window 10 - 14 min was obtained (figure 2-31). Mass spectra were obtained for the peaks appearing in this window and are summarized in table 2-12. For reference purposes, fourteen of the 21 possible dimethylquinoline isomers were analyzed by GC/MS and their retention times and mass spectra were recorded. This information is

summarized in table 2-13.

Comparison of the data in table 2-12 with those in table 2-13 reveals that the retention times and mass spectra obtained for authentic dimethylquinolines generally match the data obtained for the dimethylquinolines in the sample. In this way, it is concluded that the most abundant GC peak present in figure 2-31 is that of 2,4-dimethylquinoline. Identification of other isomers was not as successful because mass spectra and/or retention times did not match precisely. Co-elution of other material is certaintly one reason why perfect matches could not be achieved. The fact that only 14 of a possible 21 isomers of dimethylquinoline (Manske *et al.*, 1942) were available to us contributes to the difficulty of unequivocal identification of dimethylquinolines in the PANH fraction. It is also possible that many of the observed spectra in our sample are those of substituted isoquinolines which have mass spectra similar to substituted quinolines (Sample *et al.*, 1967). In any event it is clear from the observed mass spectra and similarity of retention time to authentic material that the basic component derived from coker-distillate fraction A3 (A3-basic) contains dimethylquinolines.

2.4 Conclusions

Chemical fractionation of diluted-bitumen and coker-distillate followed by bioassay using the Ames test and the Microtox test revealed that both petroleum samples were toxic. Both the acid fraction (DB-acid) and fraction A4 derived from diluted-bitumen were shown to be toxic when assessed by the Microtox test. The toxicity

associated with the acid fraction (DB-acid) was attributed to the presence of naphthenic acids since an IR spectrum for this fraction was similar to that obtained for such acids by Zenon (1986) and MacKinnon and Boerger (1986). These researchers isolated this toxic fraction from tar sands wastewater. The toxic components in diluted-bitumen fraction A4 are unknown.

Significant toxicity was observed for coker-distillate fraction A3 and A4. Both fractions revealed significant acute lethality as measured with the Microtox test and significant genotoxicity as measured with the Ames test. The Ames test revealed the presence of direct-acting mutagens in both fractions. Analysis of fraction A3 by GC/FID, GC/NPD, and HPLC/FLUOR revealed the dominate presence of PANH. HPLC/FLUOR analysis of fraction A4 revealed the presence of HPAH. Both findings were confirmed by GC/MS. Fraction A4 was found to contain substituted phenols and naphthols and fraction A3 was found to contain substituted quinolines and PANH. Further fractionation of A3 into strongly basic (A3-basic) and acid/neutral (A3-acid) material followed by further biological testing revealed enhanced genotoxicity by four fold for the basic fraction (A3-basic) and enhanced acute lethality by two fold. High resolution mass spectrometric analysis of the basic fraction revealed the presence of substituted quinolines and PANH. The presence of dimethylquinolines was confirmed by comparing the mass spectra and retention time of 14 authentic chemical standards to the sample.

The observed acute lethality for fraction A4 is reasonable since phenols are toxic to *Photobacterium phosphorium* (Lebsack *et al.*,

1981). The observed mutagenicity may be related to the presence of substituted naphthols. Substituted phenols and naphthols were detected in this sample.

The observed toxicity for fraction A3 is not unexpected since PANH have been reported to be toxic (Sidu and Blair, 1975 and Schultz *et al.*, 1982) and mutagenic (Ho *et al.*, 1979, Hirao *et al.*, 1976, and Dipple, 1976). For example, quinoline and all of its monomethyl isomers were found to be mutagens in the Ames *Salmonella* assay (Dong *et al.*, 1978). Basic PANH separated from synthetic crude oils have also been shown to be mutagenic in the Ames test (Rubin et al, 1976 and Rao *et al.*, 1979). Basic PANH which have been isolated from oil-shale, coal-liquefaction and coal-gasification processes, and elicited a mutagenic response with the Ames test, include 2-aminoanthracene, acridine, α–naphthylamine, 2,5-dimethylaniline, quinoline, 7-methylquinoline, 8-methylquinoline, 8-hydroxyquinoline, 8-aminoquinoline, and 8-nitroquinoline (Epler *et al.*, 1979 and Epler *et al.*, 1978). Derivatives of acridine were among the PANH identified in A3-basic by high resolution mass spectrometry.

Although the presence of PANH has been largely restricted to coker-distillate, as evidenced in this research, and represents less than 1% of coker-distillate on a weight basis, the extreme toxicity of these materials is cause for concern. The relative water solubility of these compounds and their extreme toxicity could cause serious environmental consequences in accidental spills. Birkholz *et al.*, (1987) found that the major water soluble components of coker-distillate fractions accidently spilled into the Athabasca river in

1982 were substituted quinoline and acridine. Further studies on the toxicity of substituted quinoline and acridine and a study on the uptake and elimination by aquatic organisms of these materials should be conducted in order to fully understand the environmental impact of these compounds if released into the environment.

2.5. TABLES

Table 2-1 Standardization of Alumina for the Class Separation of Polycyclic Aromatic Compounds

STANDARD	FRACTION	RECOVERY (%)	CLASS
n-pentacosane	A1	107	saturates
phenanthrene	A2	104	PAH/PASH[1]
benzo(g,h,i)perylene	A2	93	PAH/PASH
2-methylindole	A3	111	PANH[1]
benzo(h)quinoline	A3	97	PANH
3-aminofluoranthene	A3	81	PANH
2-naphthol	A4	87	HPAH[1]

[1] abbreviations - PAH: polycyclic aromatic hydrocarbons; PASH: polycyclic aromatic sulfur heterocycles; PANH: polycyclic aromatic nitrogen heterocycles; and HPAH: hydroxy polycyclic aromatic hydrocarbons.

Table 2-2 Mass Balance for Diluted-Bitumen

FRACTION	A1	A2	A3	A4	Acid	Total
WEIGHT %	17	23	14	6.6	0.63	62
	20	18	15	8.7	ND[1]	62
	17	21	14	12	ND	64

[1] abbreviation - ND: not determined

Table 2-3 Mass Balance for Coker-Distillate

FRACTION	A1[1]	A2[2]	A3[3]	A4[4]	Total
WEIGHT %	37	43	6.6	28	115
	39	45	6.3	18	108
	38	43	6.4	8.1	95

FRACTION	A3	
	Basic[5]	Acid/Neutral[6]
WEIGHT %	1.7 - 2.9	97.1 - 98.3

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[1] Fraction A1 contains saturates

[2] Fraction A2 contains polycyclic aromatic hydrocarbons and polycyclic aromatic sulfur heterocycles

[3] Fraction A3 contains polycyclic aromatic nitrogen heterocycles

[4] Fraction A4 contains hydroxy polycyclic aromatic hydrocarbons

[5] Basic A3 fraction contains strongly basic polycyclic aromatic nitrogen heterocycles

[6] Acid/neutral fraction of A3 contains neutral or acidic polycyclic aromatic nitrogen heterocycles

Table 2-4 Properties of Diluent Naphtha[1]

Specific gravity	0.75
Sulphur	10 ppm (w/w)
Nitrogen	1 ppm (w/w)
Bromine number	0 g/100 g

Simulated Distillation

	(TBP[2] - ^{o}C)
IBP[2]	59
5%	64
10%	83
30%	98
50%	116
70%	135
90%	160
95%	169
FBP[2]	175

[1] Data provided by Syncrude Canada Ltd.

[2] abbreviations - TBP: boiling point temperature in degrees Celsius; IBP: intial boiling point temperature in degrees Celsius; and FBP: final boiling point temperature in degrees Celsius.

Table 2-5 Properties of Coker Naphtha[1]

Density (20°C)	0.8038 g/cc
Sulphur	1.792 (wt%)
Nitrogen	175 ppm (w/w)
Bromine number	76 g/100 g

Simulated Distillation

	(TBP[2]-°C)
IBP[2]	59
5%	64
10%	83
30%	98
50%	116
70%	135
90%	160
95%	169
FBP[2]	175

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[1] Data provided by Syncrude Canada Ltd.

[2] abbreviations - TBP: boiling point temperature in degrees Celsius; IBP: intial boiling point temperature in degrees Celsius; and FBP: final boiling point temperature in degrees Celsius.

Table 2-6 Gas chromatography/mass spectrometry analysis of Coker-Distillate fraction A4

Retention Time (min)	Condensed Mass Spectra m/z(relative abundance)	Identification
6.172	94(100), 43(25), 136(15), 39(14),	phenyl acetate[1]
6.215	108(100), 107(97), 77(37), 79(35), 90(24), 80(18)	o-cresol[1]
6.558	107(100), 108(74), 79(32), 77(26), 39(16), 80(14)	p-cresol[1]
7.364	108(100), 107(36), 43(26), 77(14), 79(11), 90(10)	o- or m-methylphenyl acetate[2]
7.508	107(100), 122(40), 77(32), 79(14), 39(10), 51(10)	o-ethylphenol[1]
7.661	107(100), 122(85), 121(48), 77(30), 91(20), 39(15)	2,4-dimethylphenol[1]
7.698	107(100), 122(88), 121(42), 77(30), 79(18), 91(18)	2,5-dimethylphenol[1]
7.770	108(100), 107(42), 43(21), 150(18), 77(14), 79(12)	a methylphenyl acetate[2]
7.842	108(100), 107(48), 43(22), 77(151), 150(14), 79(8)	a methylphenyl acetate[2]
7.976	107(100), 122(76), 77(38), 43(36), 121(30), 39(21)	a dimethylphenyl acetate[2]
8.083	107(100), 122(74), 77(27), 121(26), 91(19), 79(17)	2,3-dimethylphenol[1]
8.537	107(100), 122(82), 43(26), 164(19), 77(14), 91(9)	a dimethylphenyl acetate[2]
8.880	122(100), 107(48), 121(22), 43(20), 164(18), 77(17)	a ethylphenyl acetate[2]
8.938	122(100), 107(58), 121(24), 43(22), 77(16), 91(12)	2,4-dimethylphenyl acetate[1]
9.082	122(100), 107(94), 43(26), 77(20), 164(18), 121(12)	a dimethylphenyl acetate[2]
9.265	107(100), 122(85), 43(18), 164(14), 77(14), 121(10)	a dimethylphenyl acetate[2]
9.696	122(100), 107(74), 121(25), 43(18), 164(15), 77(14)	a dimethylphenyl acetate[2]
10.268	121(100), 136(56), 43(22), 91(15), 77(14), 122(13)	a ethylmethylphenyl acetate[2]
10.460	136(100), 121(95), 43(25), 91(17), 178(17), 77(14)	a trimethylphenyl acetate[2]
10.578	107(100), 136(44), 135(27), 43(19), 134(14), 108(14)	a propylphenyl acetate[2]
13.284	144(100), 115(38), 116(24), 150(24), 43(20), 186(14),	1-naphthyl acetate[2]
13.522	144(100), 115(30), 149(28), 186(14), 43(12), 116(8)	2-naphthyl acetate[2]
14.511	158(100), 164(52), 128(22), 163(22), 43(17), 200(14)	a methylnaphthyl acetate[2]
14.868	158(100), 164(26), 163(26), 157(26), 200(20), 43(18)	a methylnaphthyl acetate[2]
15.080	158(100), 157(32), 200(19), 128(18), 43(16), 163(15)	a methylnaphthyl acetate[2]

1 identities confirmed by comparison with authentic materials

2 mass spectrum compared to authentic unacetylated substituted phenols along with deductive reasoning.

Table 2-7 Properties of Coker Gas-Oil[1]

Specific gravity	0.97
Sulphur	4.25 wt %
Nitrogen	0.24 wt %
Bromine number	31 g/100 g
Aniline point	38°C

Simulated Distillation

	(TBP[2] - °C)
IBP[2]	207
5%	247
10%	270
30%	330
50%	379
70%	433
90%	505
95%	531

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1 Data provided by Syncrude Canada Ltd.

2 abbreviations - TBP: boiling point temperature in degrees Celsius; IBP: intial boiling point temperature in degrees Celsius

Table 2-8 Properties of Bitumen[1]

Specific gravity	1.01
Sulphur	4.8 wt%
Nitrogen	0.45 wt%

Simulated Distillation

	(TBP[2] - ^{o}C)
IBP[2]	127
5%	260
10%	307
30%	437
50%	529
70%	608

1 Data provided by Syncrude Canada Ltd.

2 abbreviations - TBP: boiling point temperature in degrees Celsius; IBP: intial boiling point temperature in degrees Cesius.

Table 2-9 Microtox Results for Coker-Distillate and Diluted-Bitumen

	EC_{50}[1] (mg/L)							
FRACTION	A1[2]	A2[2]	A3[2]	A4[2]	ACID[2]	HYD[2]	A3-BASE[2]	A3-ACID[2]
Coker Dist. Aqueous[3]	3573	1393	108	49.8				
Coker Dist. Aqueous with 1,4-dioxane carrier[4]	14.8	19.0	3.04	5.89				
	31.6	18.3	4.33	16.4				
			15.2				1.50	24.2
			24.4				1.34	24.7
			20.4				3.23	15.8
Diluted Bit. Aqueous[6]	2224	1792	1994	229	172	NT[5]		
Diluted Bit. Aqueous with 1,4-dioxane	10.7	13.7	14.8	5.69				
	20.2	22.6	16.1	12.8	4.89	NT		

1 EC_{50}: that concentration which inhibits luminescence by 50 percent

2 Fraction A1 contains saturates; A2 contains polycyclic aromatic hydrocarbons and polycyclic aromatic sulfur heterocycles; A3 contains polycyclic aromatic nitrogen heterocycles; A4 contains hydroxy polycyclic aromatic hydrocarbons; ACID contains acidic compounds such as carboxylic acids; HYD contains hydrophillic compounds; A3-BASE contains strongly basic polycyclic aromatic nitrogen heterocycles, and A3-ACID contains neutral and/or acidic polycyclic aromatic nitrogen heterocycles.

3 Coker distillate fractions dissolved in water

4 Coker distillate fractions dissolved in water with carrier solvent 1,4-dioxane (150 μL solvent per 200 mL water)

5 NT means non-toxic

6 Diluted bitumen fractions dissolved in water

Table 2-10 **Ames Test Results for Coker-Distillate and Diluted-Bitumen**

Coker-distillate - A1[1]	NO EFFECT	Max. appl. /plate = 4.4 mg
Coker-distillate- A2[1]	NO EFFECT	Max. appl. /plate = 6.1 mg
Coker-distillate - A3[1]	MUTAGENIC DIRECT ACTING	10 mg = 464 - 776 rev r^2 = 0.83 - 0.86
Coker-distillate - A4[1]	MUTAGENIC DIRECT ACTING	1.0 mg = 150 rev r^2 = 0.86
Coker-distillate -A3-Basic[1]	MUTAGENIC ENZYME ACTIVATION REQ'D.	1.0 mg = 2390 rev r^2 = 0.98
Coker-distillate -A3-Acidic[1]	MUTAGENIC DIRECT ACTING	10 mg = 670 rev r^2 = 0.96
Diluted-bitumen - A1	NO EFFECT	Max. appl. /plate = 0.968 mg
Diluted-bitumen - A2	NO EFFECT	Max. appl. /plate = 1.4 mg
Diluted-bitumen - A3	NO EFFECT	Max. appl. /plate = 0.66 mg
Diluted-bitumen - A4	NO EFFECT	Max. appl. /plate = 0.58 mg
Diluted-bitumen - Acid[1]	TOXIC	Max. appl. /plate = 0.14 mg
Benzo(a)pyrene	MUTAGENIC ENZYME ACTIVATION REQ'D.	1.0 μg = 171 - 190 rev corr. coef. = 0.97
Spontaneous controls		12 - 24 rev

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1 Fraction A1 contains saturates; A2 contains polycyclic aromatic hydrocarbons and polycyclic aromatic sulfur heterocycles; A3 contains polycyclic aromatic nitrogen heterocycles; A4 contains hydroxy polycyclic aromatic hydrocarbons; A3-Basic contains strongly basic polycyclic aromatic nitrogen heterocycles; A3-Acid contains neutral and/or acidic polycyclic aromatic nitrogen heterocycles and Acid contains acidic compounds such as carboxylic acids

Table 2-11 Gas chromatography/high resolution mass spectrometry analysis of Coker-distillate basic compounds in fraction A3

Scan #	Condensed mass spectrum m/z(relative abundance)	Formula	Compound Identification
85	157(100), 156(25), 162(16), 142(14), 115(10), 158(10), 171(10), 170(9)	$C_{11}H_{11}N$	a dimethylquinoline[1]
165	171(100), 170(30), 162(25), 156(23), 177(19), 172(15), 176(12), 128(5)	$C_{12}H_{13}N$	a trimethylquinoline[1]
174	171(100), 170(20), 172(15), 156(13), 128(8), 162(5), 86(5), 115(3)	$C_{12}H_{13}N$	a trimethylquinoline[1]
188	171(100), 170(18), 156(15), 172(12), 162(10), 128(8), 86(5), 115(3)	$C_{12}H_{13}N$	a trimethylquinoline[1]
274	184(100), 185(62), 171(35), 157(25), 162(10), 170(10), 115(9), 163(7)	$C_{13}H_{15}N$	a 2-ethyl-dimethylquinoline or a 8-ethyl-dimethylquinoline[2]
291	184(100), 185(72), 157(25), 171(21), 162(18), 163(13), 128(10), 115(8)	$C_{13}H_{15}N$	a 2-ethyl-dimethylquinoline or a 8-ethyl-dimethylquinoline[2]
299	184(100), 185(80), 17(25), 170(22), 157(22), 149(15), 156(13), 163(10)	$C_{13}H_{15}N$	a 2-ethyl-dimethylquinoline a 8-ethyl-dimethylquinoline[2]
316	191(100), 190(75), 171(35), 176(30), 163(18), 192(15), 177(10), 170(10)	$C_{14}H_9N$	PANH Figure 2-26(a)[3]
341	185(100), 170(22), 184(20), 186(15), 171(15), 168(10), 128(8), 115(5)	$C_{13}H_{15}N$	a tetramethylquinoline[2]
361	185(100), 183(35), 184(25), 170(22), 162(15), 182(12), 163(11), 168(10)	$C_{13}H_{15}N$	a tetramethylquinoline[2]
389	185(100), 184(45), 170(30), 162(20), 171(18), 186(15), 191(10), 163(10)	$C_{13}H_{15}N$	a tetramethylquinoline[2]

1 Structure determined from the literature (Eight Peak Index of Mass Spectra, 1983)

2 Structure determined from the fragmentation of alkylquinolines (Draper and MacLean, 1968)

3 PANH: a polycyclic aromatic nitrogen-containing heterocyclic compound; structure deduced from rings plus double bonds forumula (McLafferty, 1980)

396	185(100), 184(42), 198(25), 199(23), 170(22), 171(16), 162(12), 186(12)	$C_{13}H_{15}N$	a tetramethylquinoline[2]
408	185(100), 184(50), 162(25), 170(25), 171(17), 163(16), 176(10), 128(10)	$C_{13}H_{15}N$	a tetramethylquinoline[2]
423	198(100), 199(75), 171(40), 162(25), 185(16), 170(16), 163(15), 184(15)	$C_{14}H_{17}N$	a 2-ethyl-trimethylquinoline or a 8-ethyl-trimethylquinoline[2]
457	198(100), 199(85), 162(42), 163(30), 171(30), 185(25), 182(20), 184(18)	$C_{14}H_{17}N$	a 2-ethyl-trimethylquinoline or a 8-ethyl-trimethylquinoline[2]
463	185(100), 184(22), 170(16), 186(15), 162(11), 128(10), 115(5(, 163(5)	$C_{13}H_{15}N$	a tetramethylquinoline[2]
470	199(100), 184(75), 185(25), 162(25), 200(20), 163(20), 168(15), 191(10)	$C_{14}H_{17}N$	a 4-ethyl-trimethylquinoline[2]
479	198(100), 199(75), 162(38), 168(30), 184(27), 185(25), 163(25), 171(25)	$C_{14}H_{17}N$	a 2-ethyl-trimethylquinoline or a 8-ethyl-trimethylquinoline[2]
498	184(100), 199(75), 162(37), 163(24), 196(25), 185(25), 198(24), 197(20)	$C_{14}H_{17}N$	a ethyl-trimethylquinoline[2] not 2-, 4-, or 8-ethyl
505	199(100), 184(65), 162(39), 185(38), 198(38), 163(30), 200(18), 191(13)	$C_{14}H_{17}N$	a 4-ethyl-trimethylquinoline[2]
517	183(100), 199(75), 198(70), 162(60), 197(58), 182(55), 184(36), 196(25)	$C_{14}H_{17}N$	a 2,?-diethyl-methylquinoline or a 8,?-diethyl-methylquinoline[2]
539	199(100), 184(38), 198(27), 162(23), 185(17), 200(16), 163(13), 128(10)	$C_{14}H_{17}N$	a pentamethylquinoline[2]
554	198(100), 199(70), 171(25), 200(10), 162(10), 184(7), 196(7), 163(7)	$C_{14}H_{17}N$	a 2-ethyl-trimethylquinoline or a 8-ethyl-trimethylquinoline[2]
590	199(100), 184(42), 198(42), 163(35), 183(15), 196(15), 197(15), 200(14)	$C_{14}H_{17}N$	a pentamethylquinoline[2]
616	198(100), 199(96), 213(38), 185(38), 212(35), 162(28), 196(25), 171(23)	$C_{14}H_{17}N$ + $C_{15}H_{19}N$	C_5-quinoline[4] C_6-quinoline[5]

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2 Structure determined from the fragmentation of alkylquinolines (Draper and MacLean, 1968)

4 Mixture, chemical structure difficult to interpret, C_5 indicates a substitution pattern totals C_5H_{11} (i.e. C_5H_{11}; 2 x C_2H_5 + CH_3; C_2H_5 + C_3H_7; 5 x CH_3; etc)

5 C_6 indicates a substitution pattern which totals C_6H_{13} (i.e. 6 x CH_3; 3 x C_2H_5; 2 x C_3H_7; etc)

621	212(100), 213(78), 185(25), 197(22), 179(20), 196(15), 163(14), 214(12)	$C_{15}H_{19}N$	a 2-ethyl-tetramethylquinoline or a 8-ethyl-tetramethylquinoline[2]
661	198(100), 196(70), 162(50), 185(42), 197(40), 163(30), 213(25), 199(22)	$C_{15}H_{19}N$	a 8-propyl-ethyl-methylquinoline[2]
668	197(100), 196(32), 199(28), 198(25), 162(20), 163(16), 182(15), 180(10)	$C_{14}H_{15}N$	a trimethyl-aza-acenaphthene[3]
704	211(100), 162(97), 57(78), 196(63), 83(62), 163(62), 195(52), 55(50)	$C_{15}H_{17}N$	a C_4-aza-acenaphthene[3]
708	193(100), 195(55), 162(52), 194(50), 197(40), 196(32), 163(32), 192(30	$C_{14}H_{11}N$ + $C_{15}H_{17}N$	a methylacridine[1] a C_4-aza-acenaphthene[3]
731	193(100), 162(45), 192(38), 163(32), 191(27), 194(26), 196(17), 197(15)	$C_{14}H_{11}N$	a methylacridine[1]
768	209(100), 194(28), 208(25), 210(20), 162(12), 105(7), 104(6), 165(5)	$C_{15}H_{15}N$	PANH Figure 2-26(b) C_3-substituted[3]
938	227(100), 226(28), 228(22), 114(12), 163(10), 228(10), 209(10), 207(10)	$C_{17}H_9N$	PANH Figure 2-26(c)[3]
978	227(100), 163(32), 226(18), 228(15), 222(10), 221(10), 65(10), 57(10)	$C_{17}H_9N$	PANH Figure 2-26(c)[3]
1022	223(100), 227(98), 221(85), 162(55), 226(35), 208(35), 222(25), 220(18)	$C_{17}H_9N$ +	PANH Figure 2-26(c)[3]
		$C_{16}H_{17}N$	PANH Figure 2-26(b) C_4-substituted[3]
1029	221(100), 220(35), 222(23), 227(15), 163(15), 111(10), 206(8), 102(8)	$C_{16}H_{15}N$	a C_3-acridine[3]
1161	233(100), 234(25), 162(20), 218(12), 232(10), 117(8), 235(5), 154(5)	$C_{17}H_{15}N$	PANH Figure 2-26(a) C_3-substituted[3]

3 PANH: a polycyclic aromatic nitrogen-containing heterocyclic compound; structure deduced from rings plus double bonds formula (McLafferty, 1980)

1 Structure determined from the literature (Eight Peak Index of Mass Spectra, 1983)

1279	247(100), 162(28), 248(23), 163(20), 73(20), 241(18), 207(12), 246(9)	$C_{18}H_{17}N$	PANH Figure 2-26(a) C_4-substituted[3]
1287	247(100), 162(38), 73(25), 163(22), 254(22), 221(20), 207(18), 248(15)	$C_{18}H_{17}N$	PANH Figure 2-26(a) C_4-substituted[3]

3 PANH: a polycyclic aromatic nitrogen-containing heterocyclic compound; structure deduced from rings plus double bonds formula (McLafferty, 1980)

Table 2-12 Mass Spectra of Dimethylquinolines Present in Coker-Distillate Fraction A3 - Basic Component

Retention Time (min)	Condensed Mass Spectra m/z (relative abundance)
10.955	157(100), 156(44), 39(28), 41(25), 77(24), 43(23)
11.499	157(100), 156(49), 39(18), 115(17), 142(13), 77(12)
11.780	157(100), 156(27), 115(20), 39(14), 158(12), 142(10)
11.884	157(100), 156(40), 115(28), 43(28), 39(24), 142(20)
11.953	157(100), 156(43), 142(25), 77(20), 39(18), 63(15)
11.967	157(100), 156(44), 142(33), 77(19), 39(18), 51(14)

Table 2-13 Condensed Mass Spectra of Dimethylquinolines

Retention Time (min)	Condensed Mass Spectra m/z (relative abundance)	Isomer
10.911	157(100), 156(38), 142(15), 115(12), 158(11), 63(9)	2,8-DMQ
11.444	157(100), 156(37), 142(14), 115(14), 142(14), 158(11)	2,7-DMQ
11.547	157(100), 142(51), 156(44), 77(13), 154(12), 158(12)	6,8-DMQ
11.674	157(100), 156(44), 142(26), 128(13), 158(11), 77(11)	3,8-DMQ
11.697	157(100), 156(25), 115(18), 158(12), 142(9), 39(8)	2,4-DMQ
11.872	157(100), 156(69), 142(36), 154(18), 77(16), 51(12)	7,8-DMQ
11.891	157(100), 156(41), 142(20), 77(14), 158(12), 128(10)	4,8-DMQ
11.926	142(100), 157(63), 115(35), 156(21), 143(11), 89(11),	3-ETHQ
12.108	157(100), 156(47), 142(29), 128(15), 51(12), 158(11)	3,7-DMQ
12.138	157(100), 156(48), 142(27), 128(13), 158(12), 63(11)	3,6-DMQ
12.282	157(100), 142(58), 156(49), 154(12), 77(12), 158(11)	5,7-DMQ
12.342	157(100), 156(42), 142(22), 158(11), 77(11), 128(10)	4,6-DMQ
12.348	157(100), 156(44), 142(26), 128(14), 158(11), 51(11)	3,5-DMQ
12.488	157(100), 156(50), 142(57), 77(13), 154(12), 141(12)	6,7-DMQ
12.677	142(100), 157(97), 156(42), 154(14), 141(14), 77(14)	5,6-DMQ

2.6. FIGURES

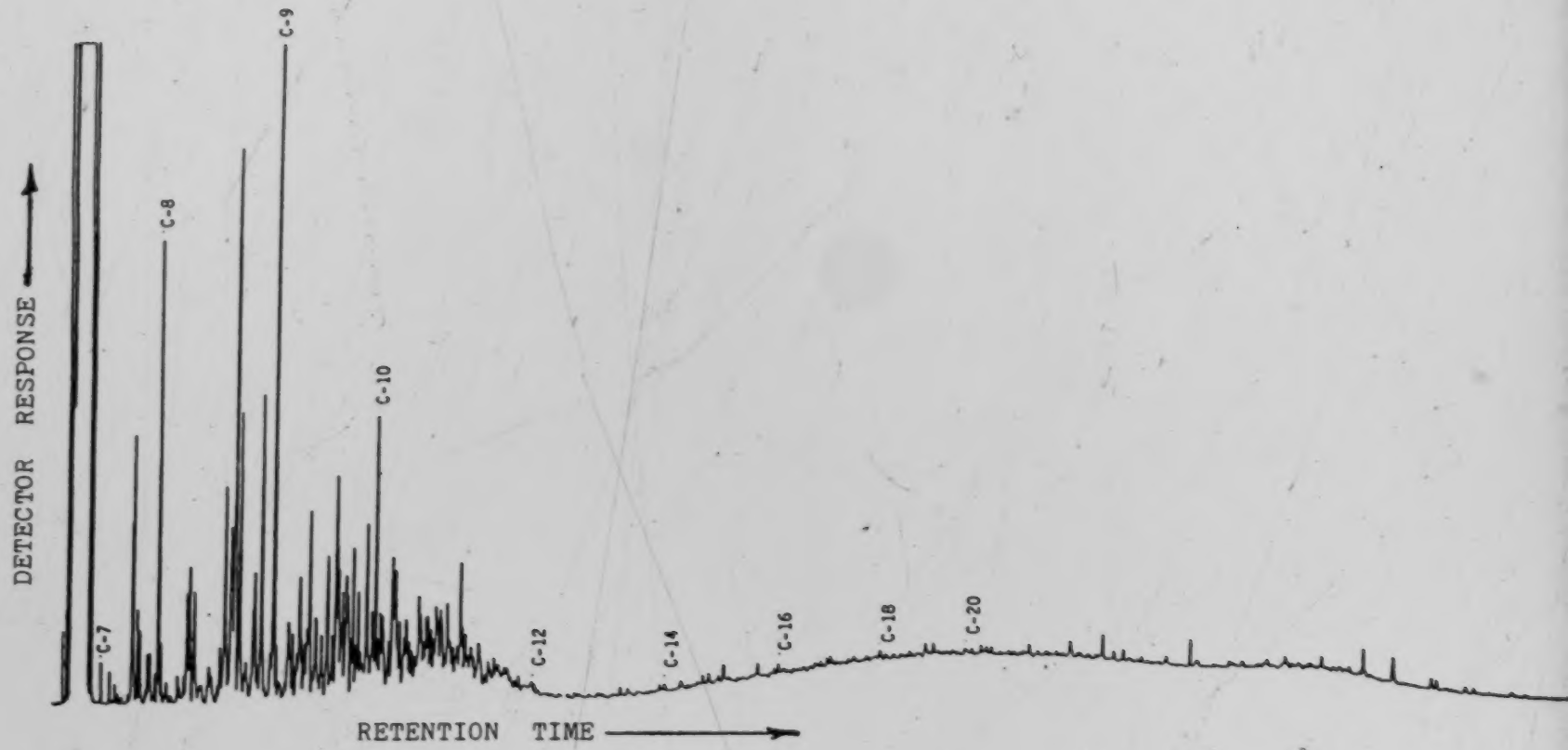


Figure 2-1 DILUTED BITUMEN - HEXANE FRACTION
31 ug Att 32
FID

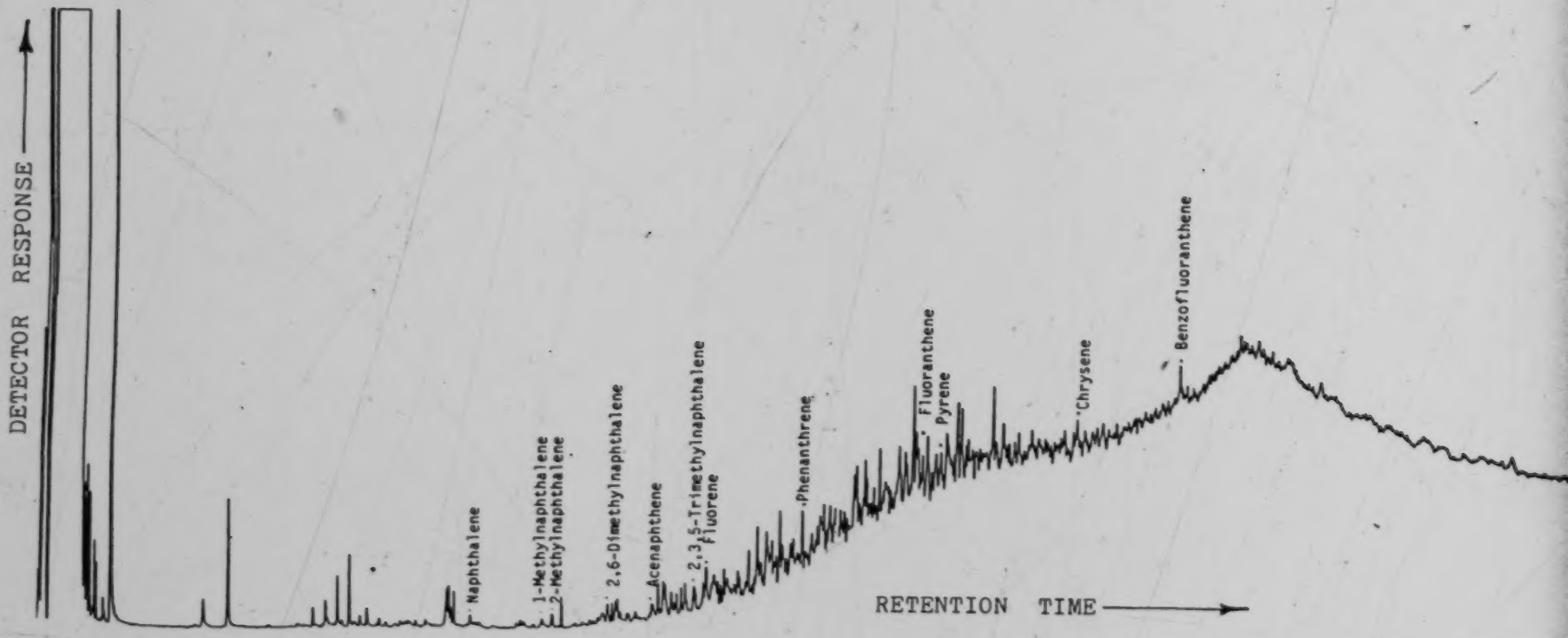


Figure 2-2 DILUTED BITUMEN - BENZENE FRACTION
38 ug Att 2
FID

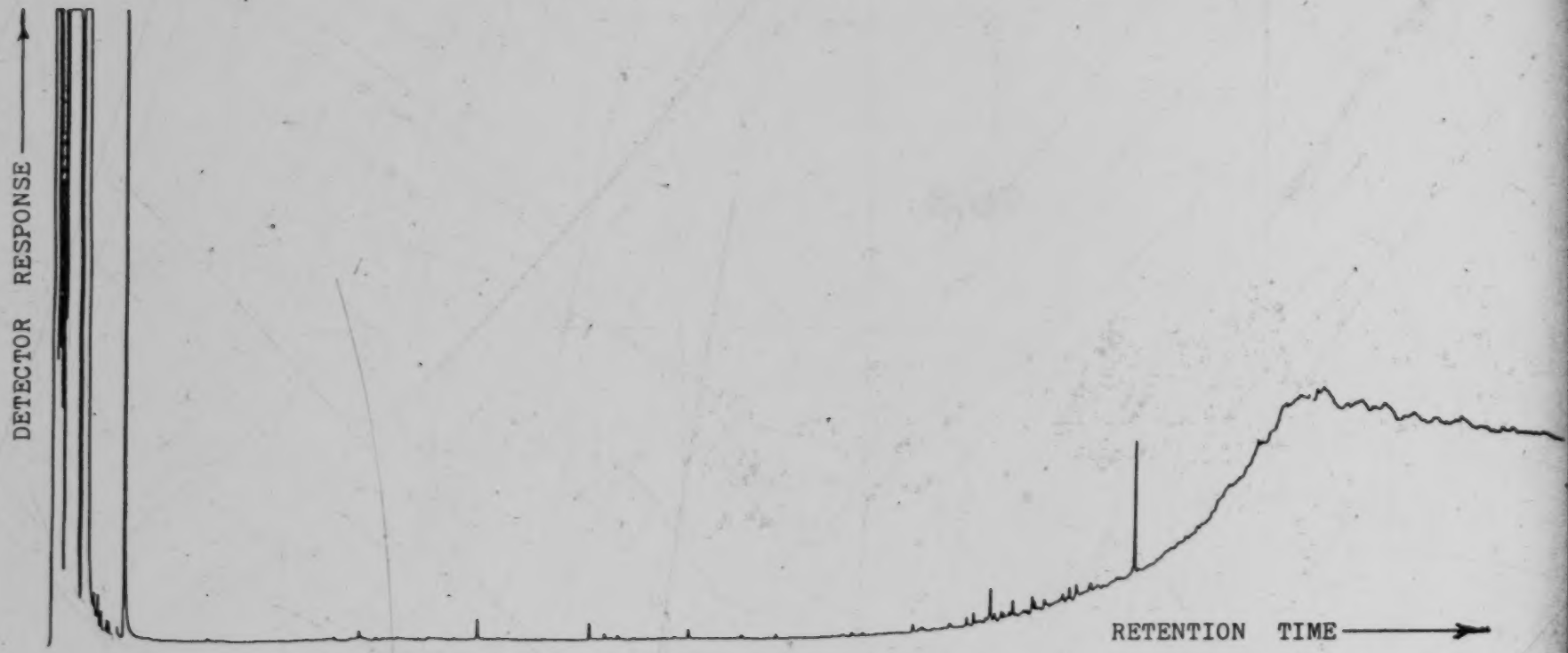


Figure 2-3 DILUTED BITUMEN - CHLOROFORM FRACTION
31 ug Att 2
FID

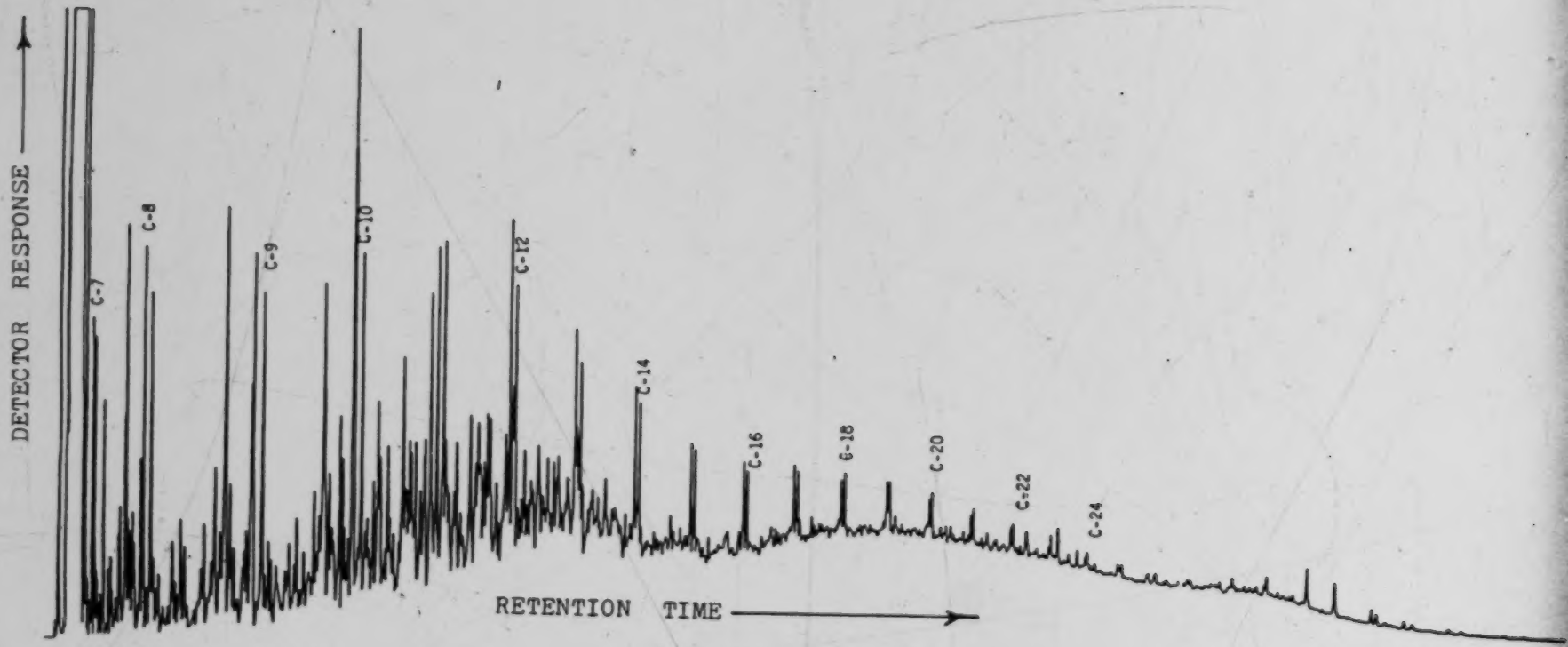


Figure 2-4 COKER DISTILLATE - HEXANE FRACTION
117 ug Att 64
FID

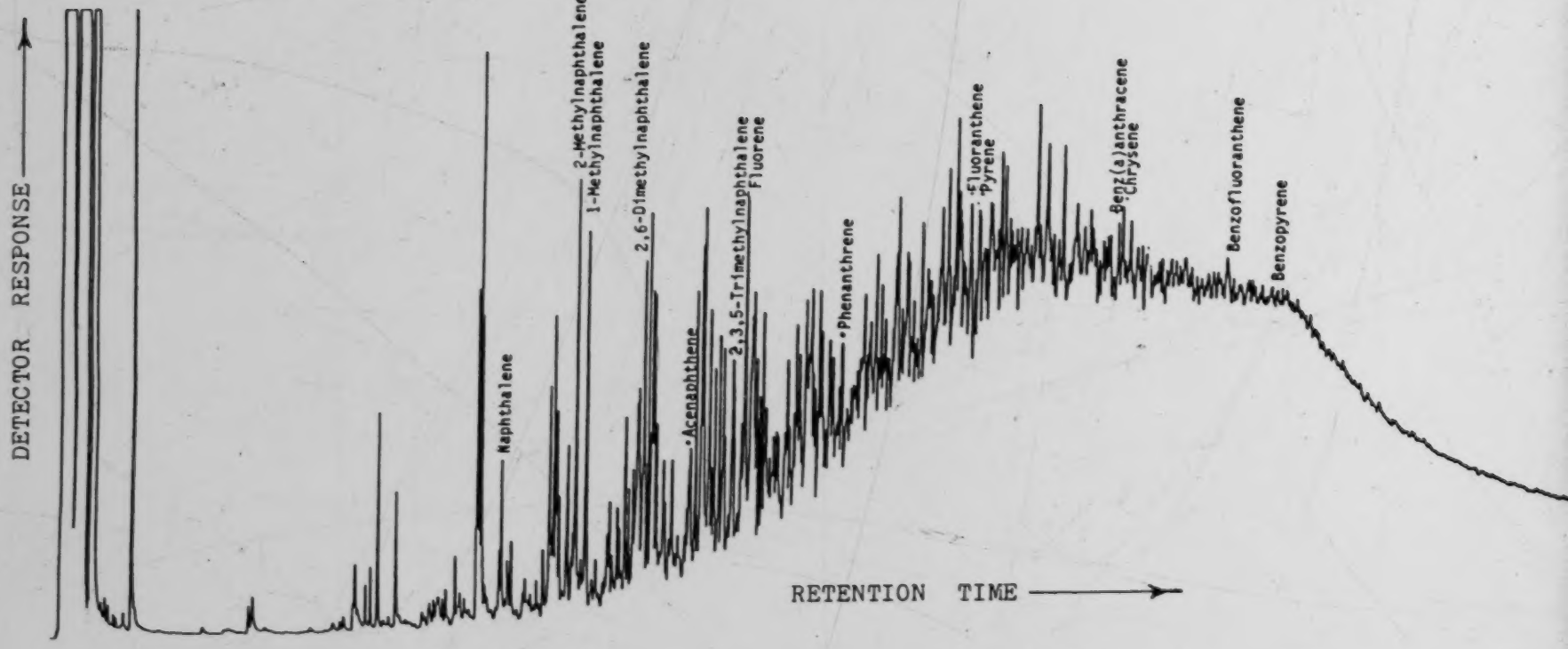


Figure 2-5 COKER DISTILLATE - BENZENE FRACTION
13.2 ug Att 2
FID

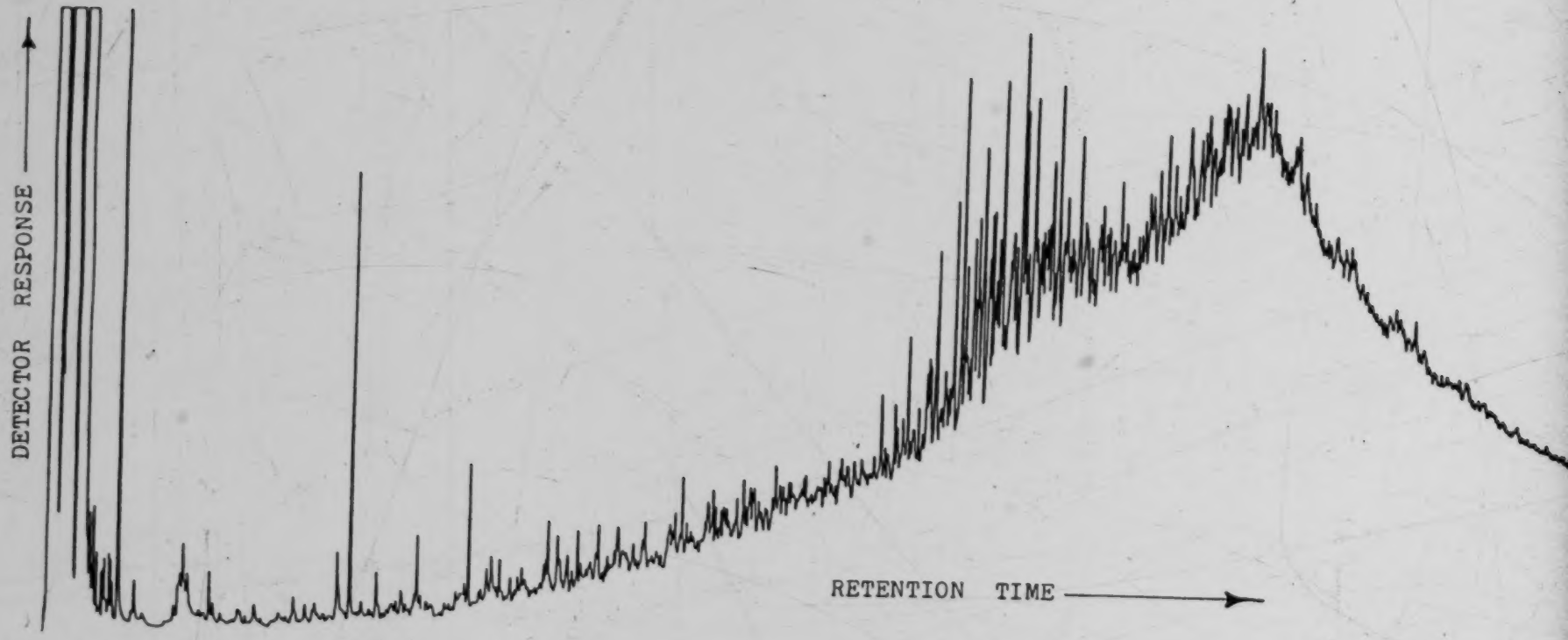


Figure 2-6 COKER DISTILLATE - CHLOROFORM FRACTION
20 ug Att 2
FID

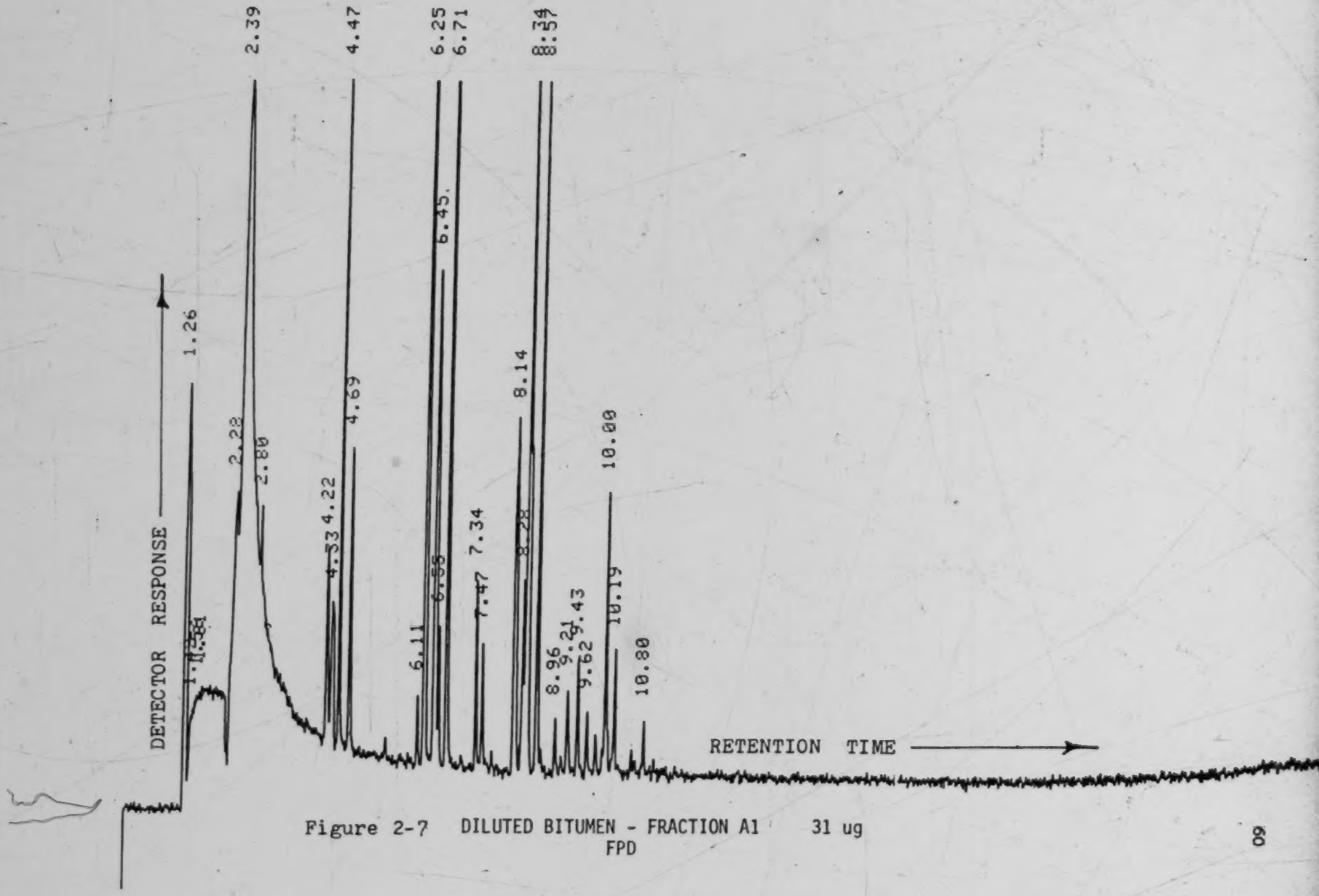


Figure 2-7 DILUTED BITUMEN - FRACTION A1 31 ug
FPD

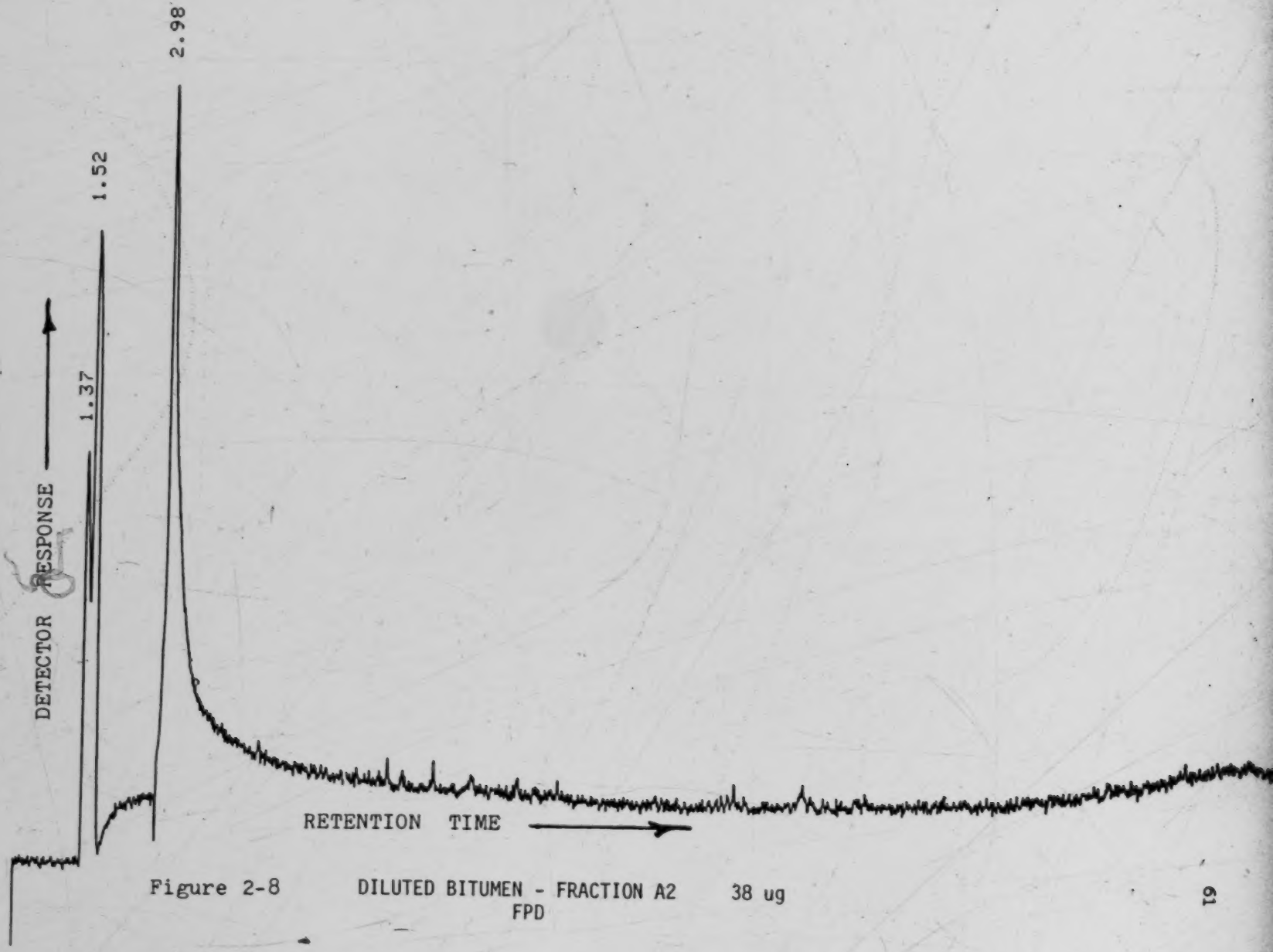


Figure 2-8 DILUTED BITUMEN - FRACTION A2 38 ug
FPD

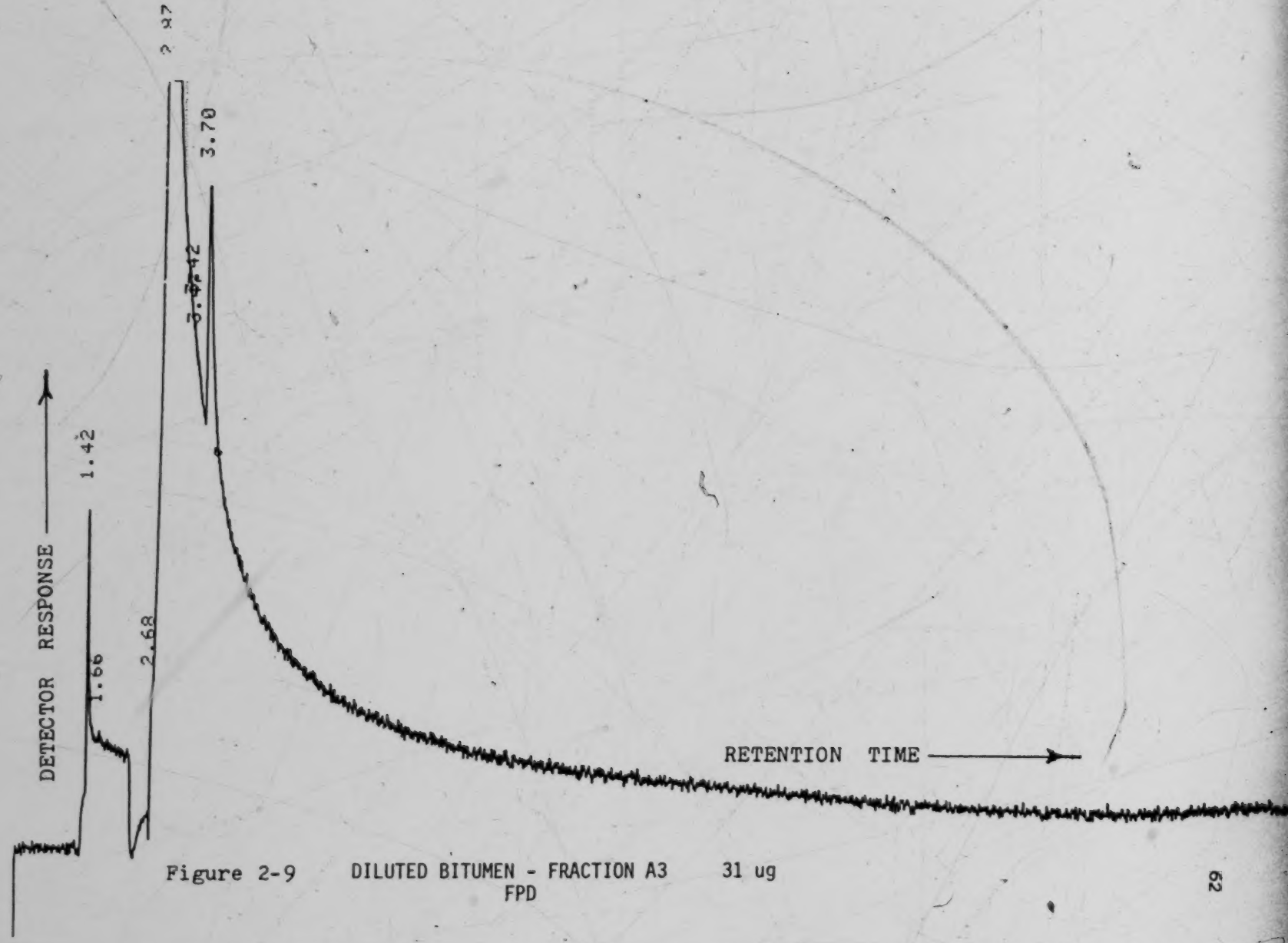


Figure 2-9 DILUTED BITUMEN - FRACTION A3 31 ug
FPD

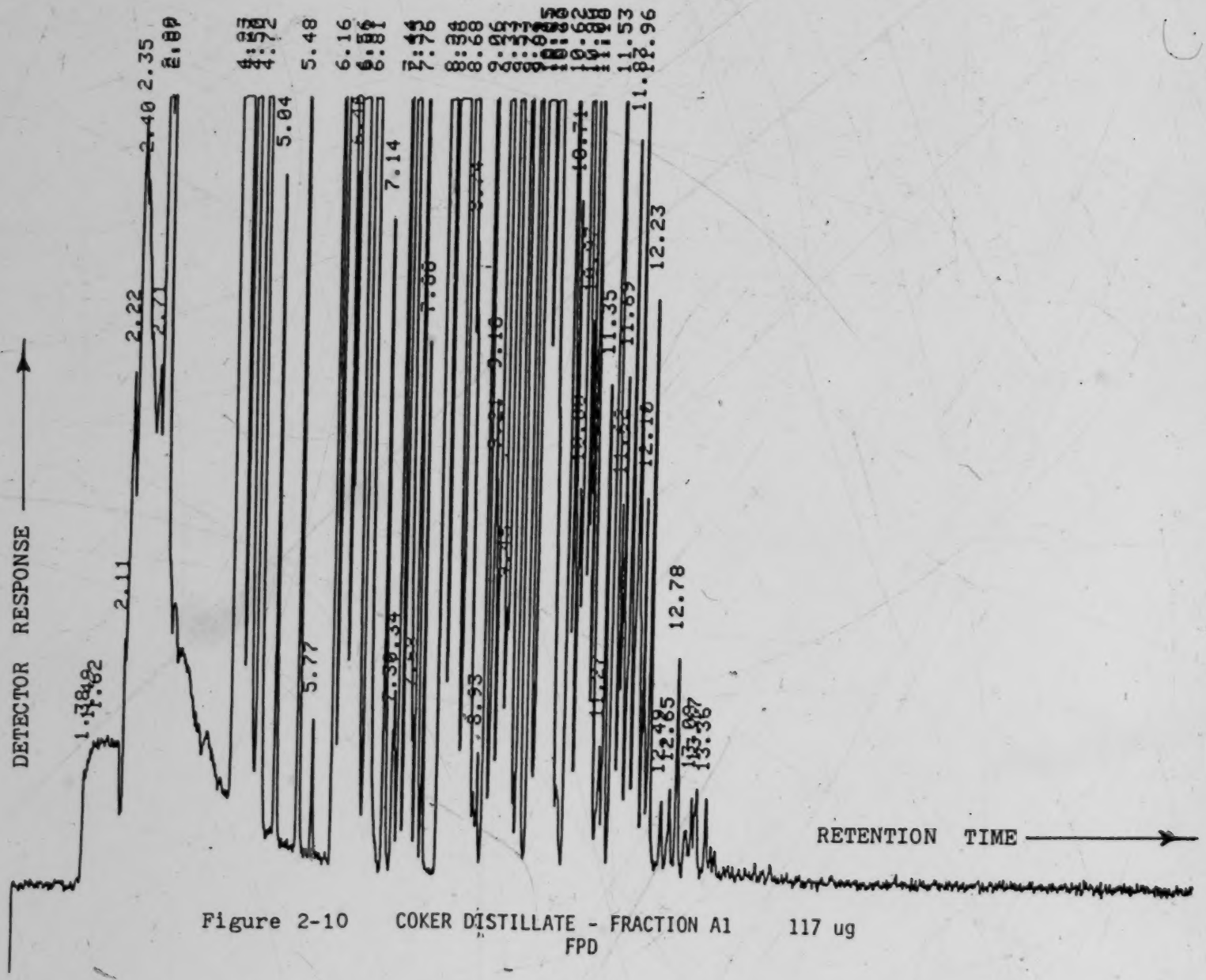


Figure 2-10 COKER DISTILLATE - FRACTION A1 117 ug
FPD

Figure 2-11 COKER DISTILLATE - FRACTION A2 132 ug
FPD

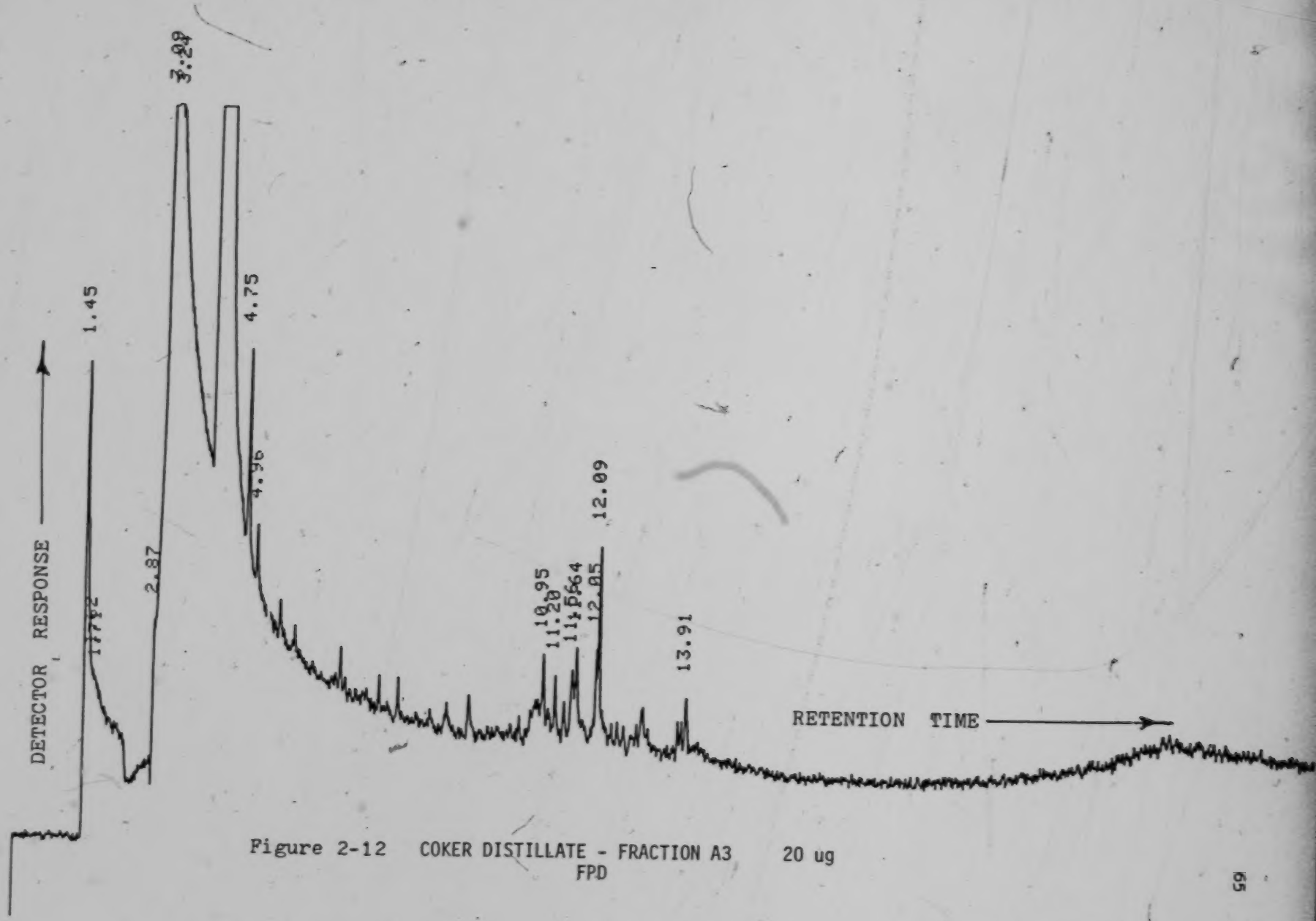


Figure 2-12 COKER DISTILLATE - FRACTION A3 20 ug
FPD

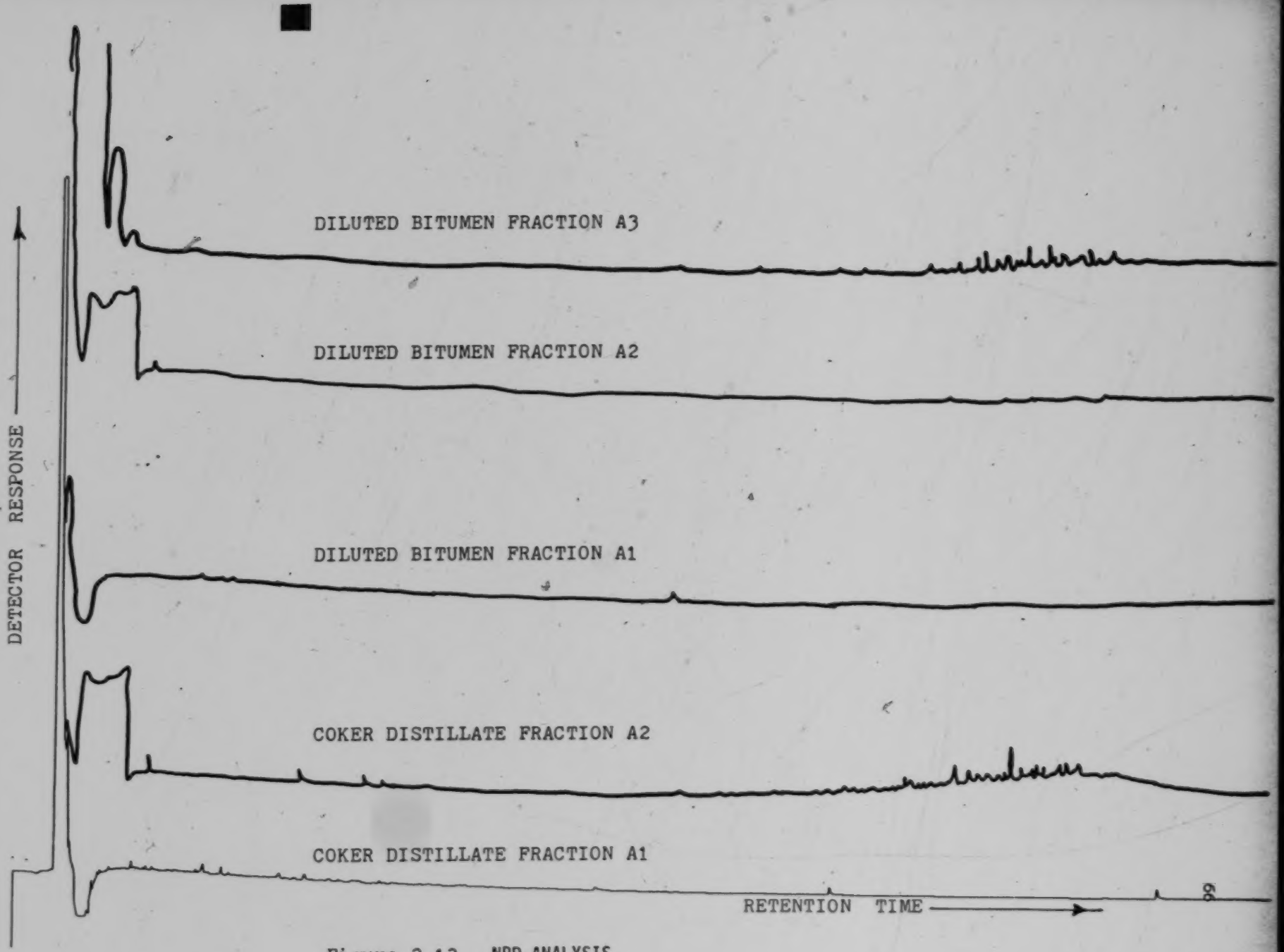


Figure 2-13 NPD ANALYSIS

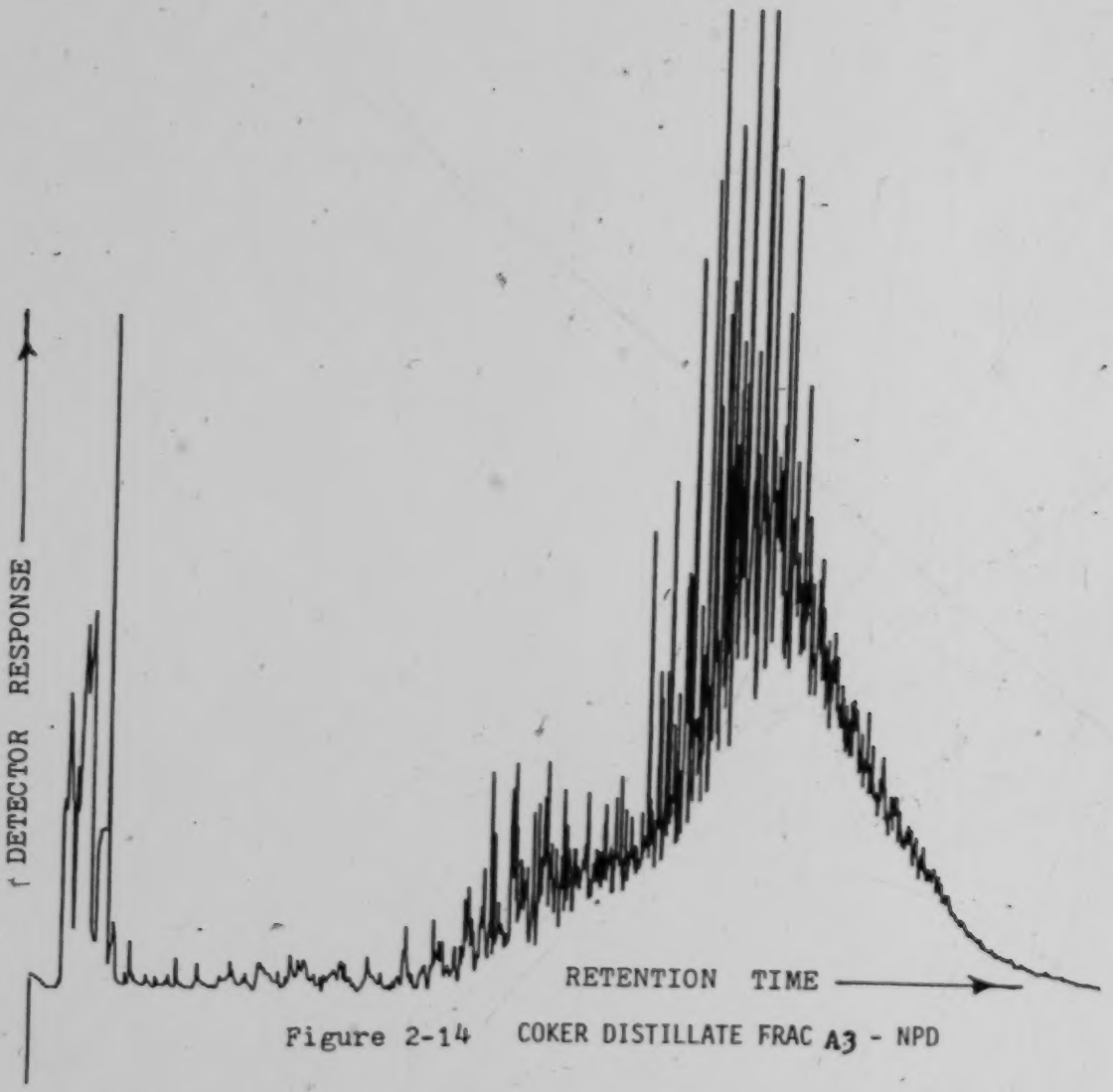


Figure 2-14 COKER DISTILLATE FRAC A3 - NPD

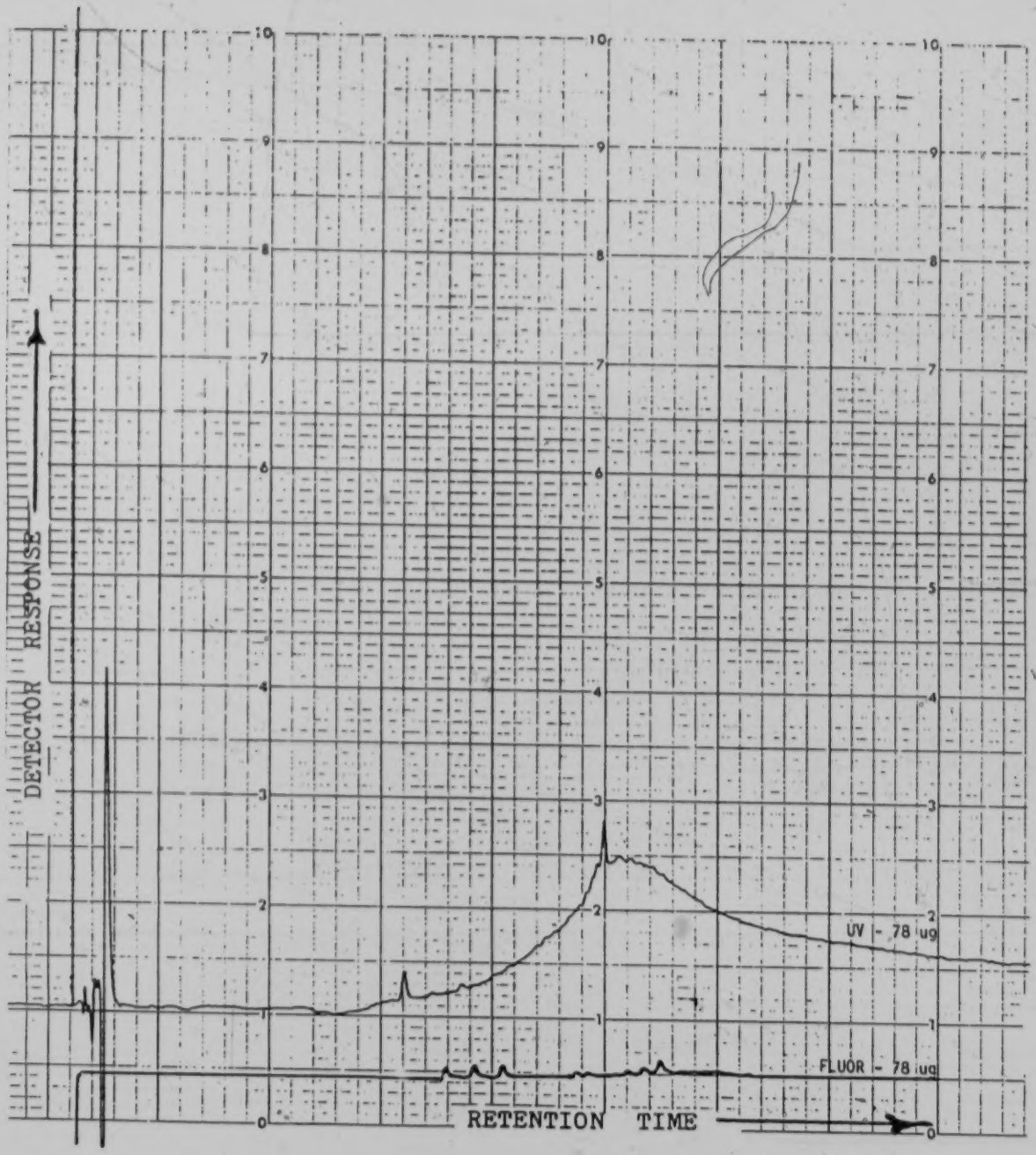


Figure 2-15 Diluted Bitumen Fraction A1
HPLC Analysis

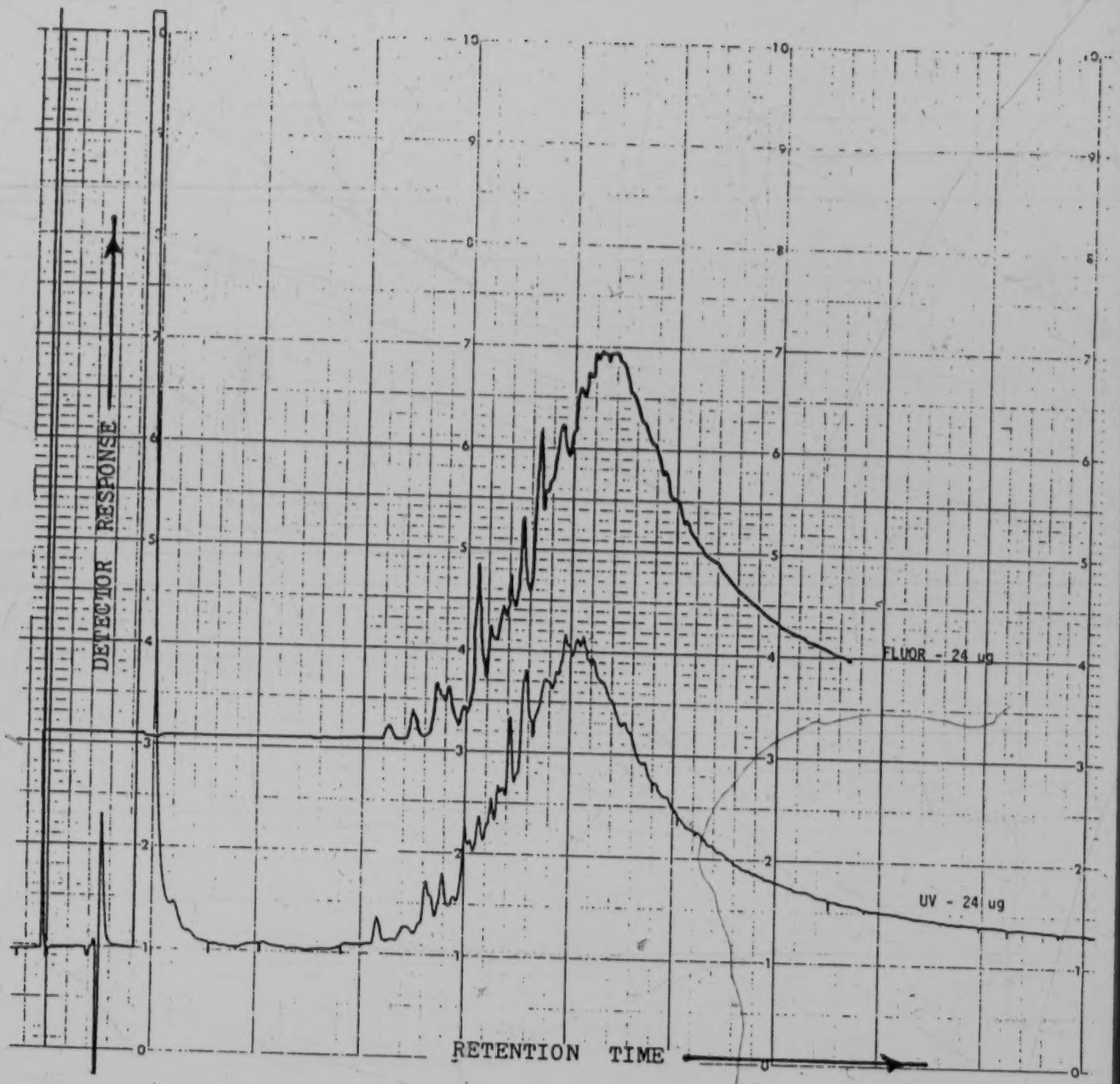


Figure 2-16 Diluted Bitumen Fraction A2
HPLC Analysis

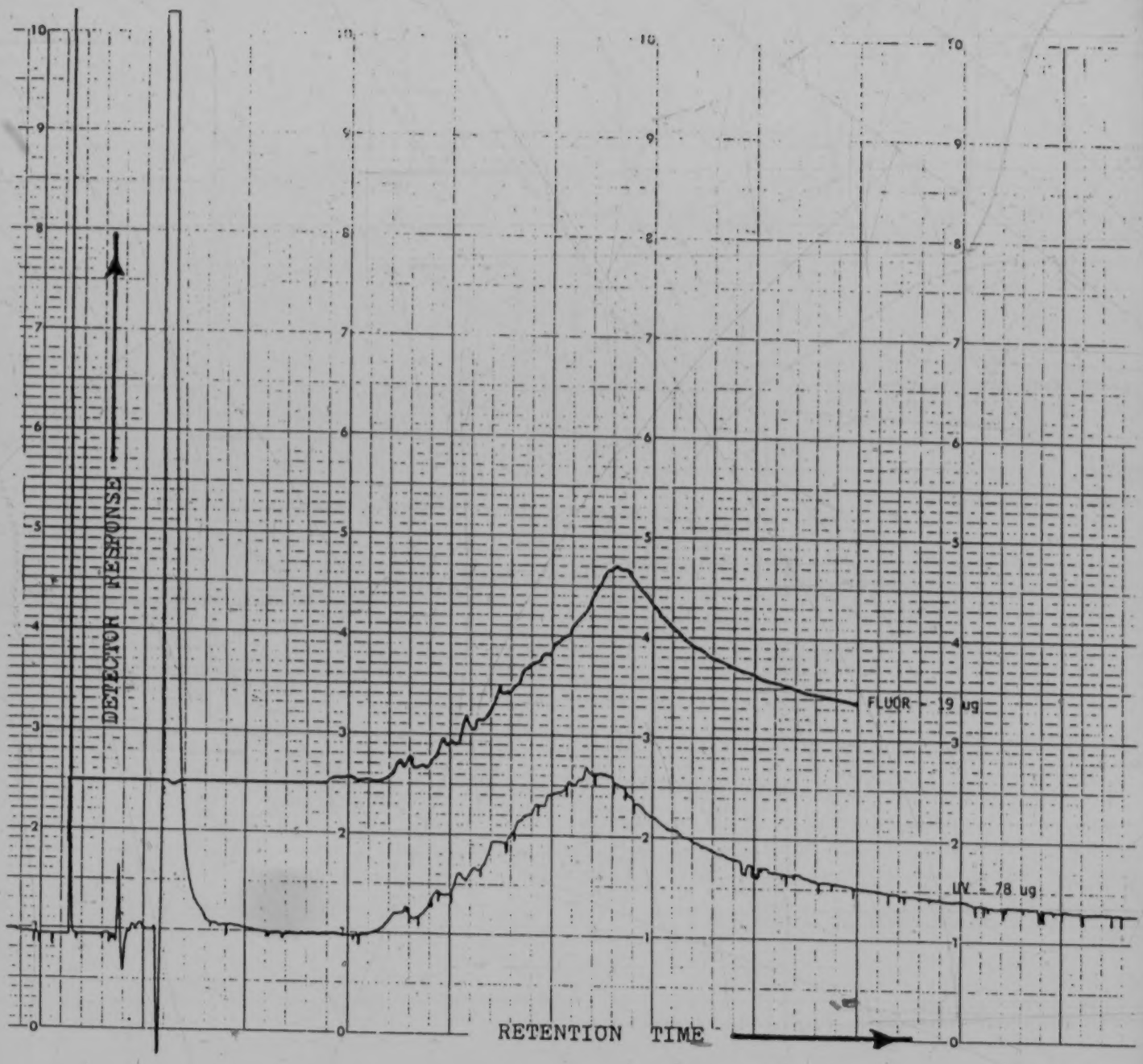


Figure 2-17 Diluted Bitumen Fraction A3
HPLC Analysis

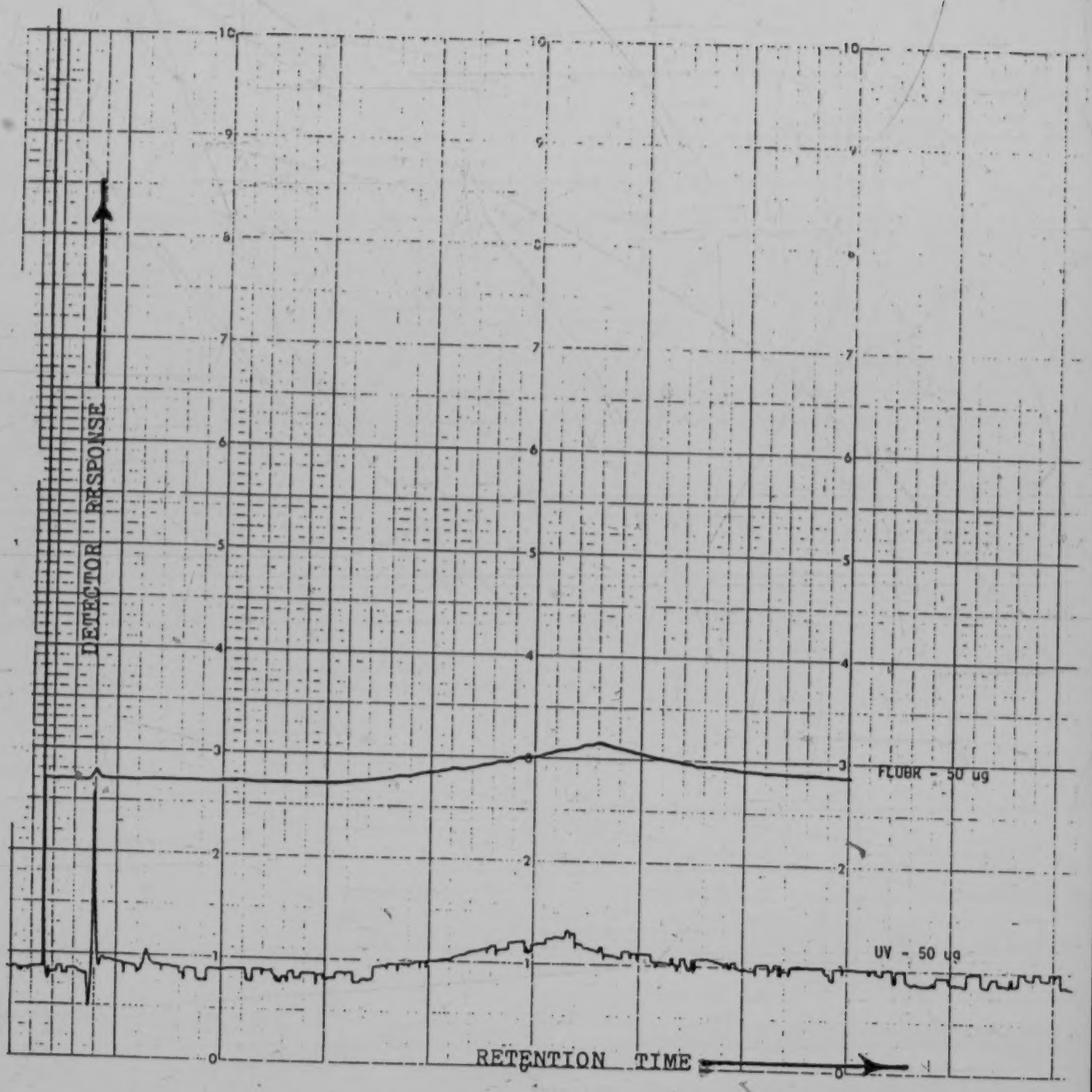


Figure 2-18 Diluted Bitumen Fraction A4
HPLC Analysis

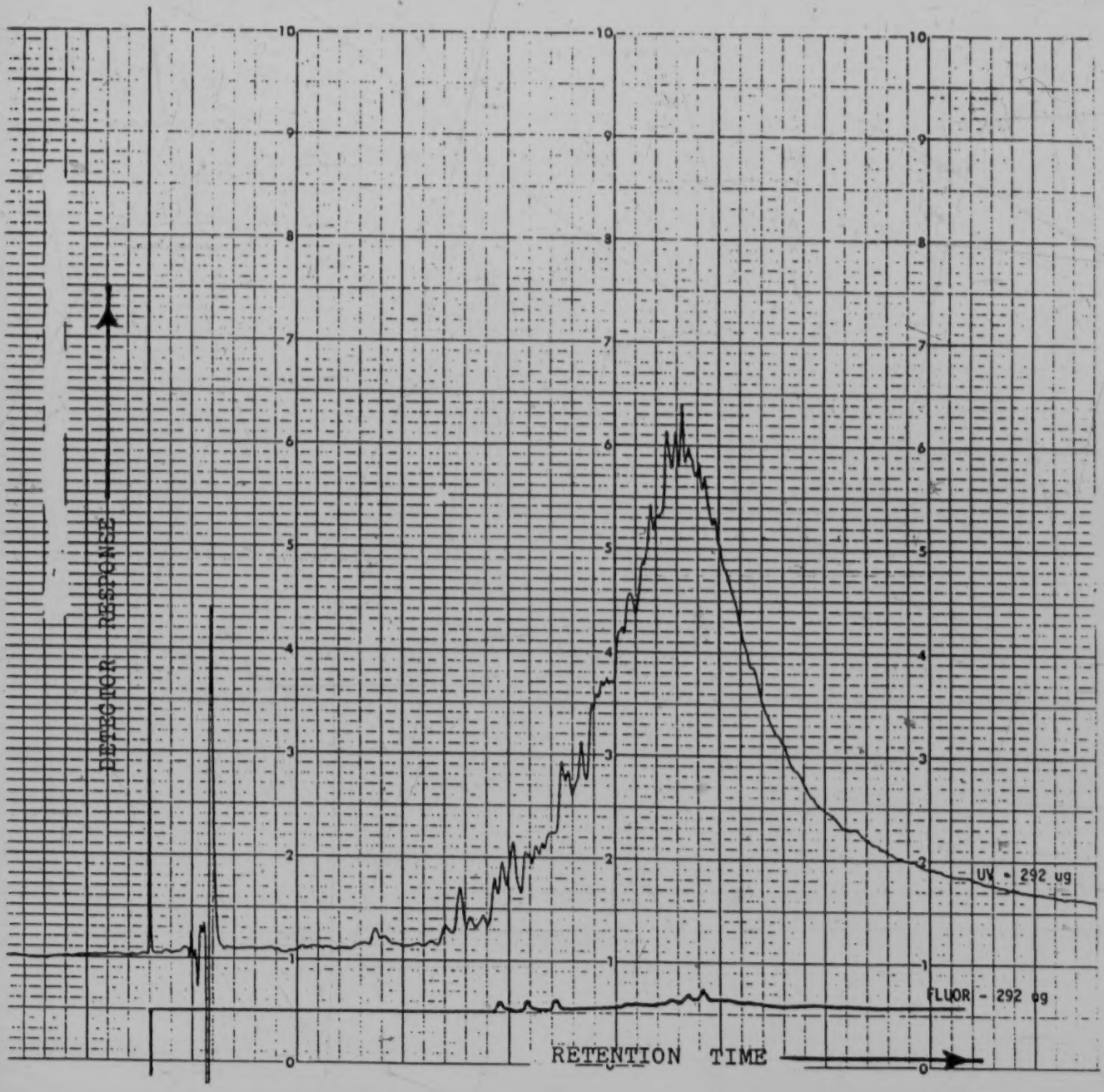


Figure 2-19 Coker Distillate Fraction A1
HPLC Analysis

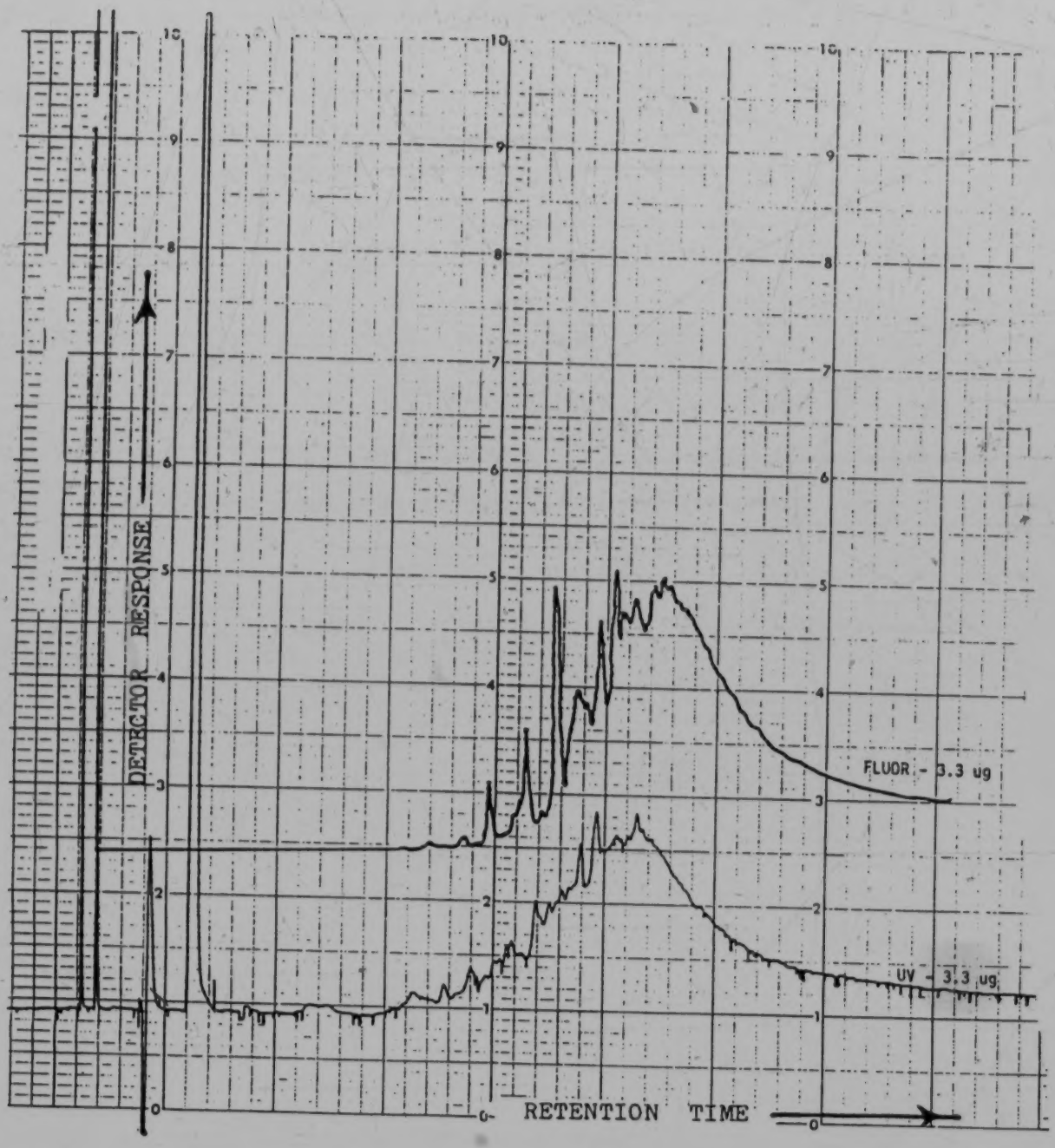


Figure 2-20 Coker Distillate Fraction A2
HPLC Analysis

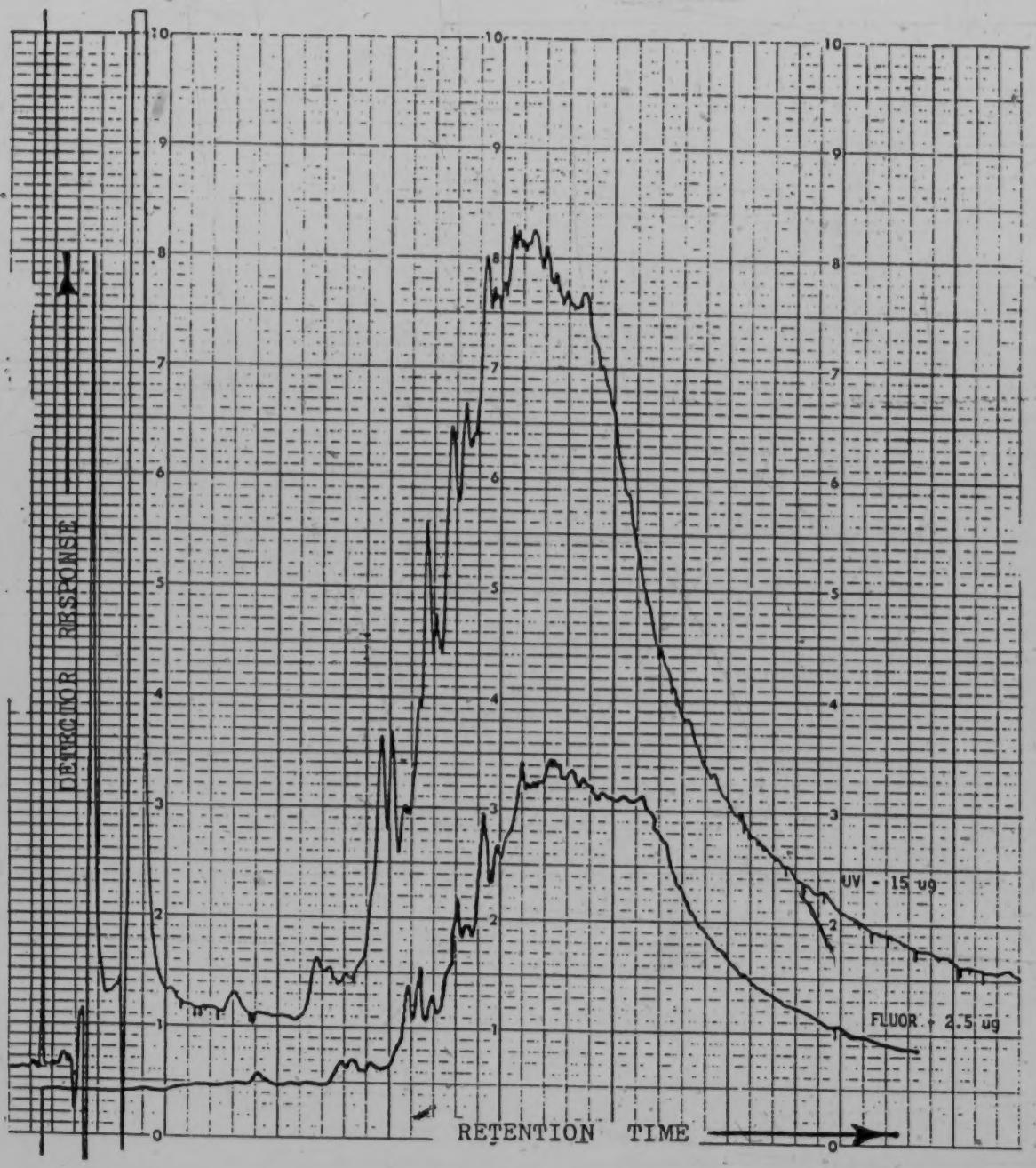


Figure 2-21 Coker Distillate Fraction A3
HPLC Analysis

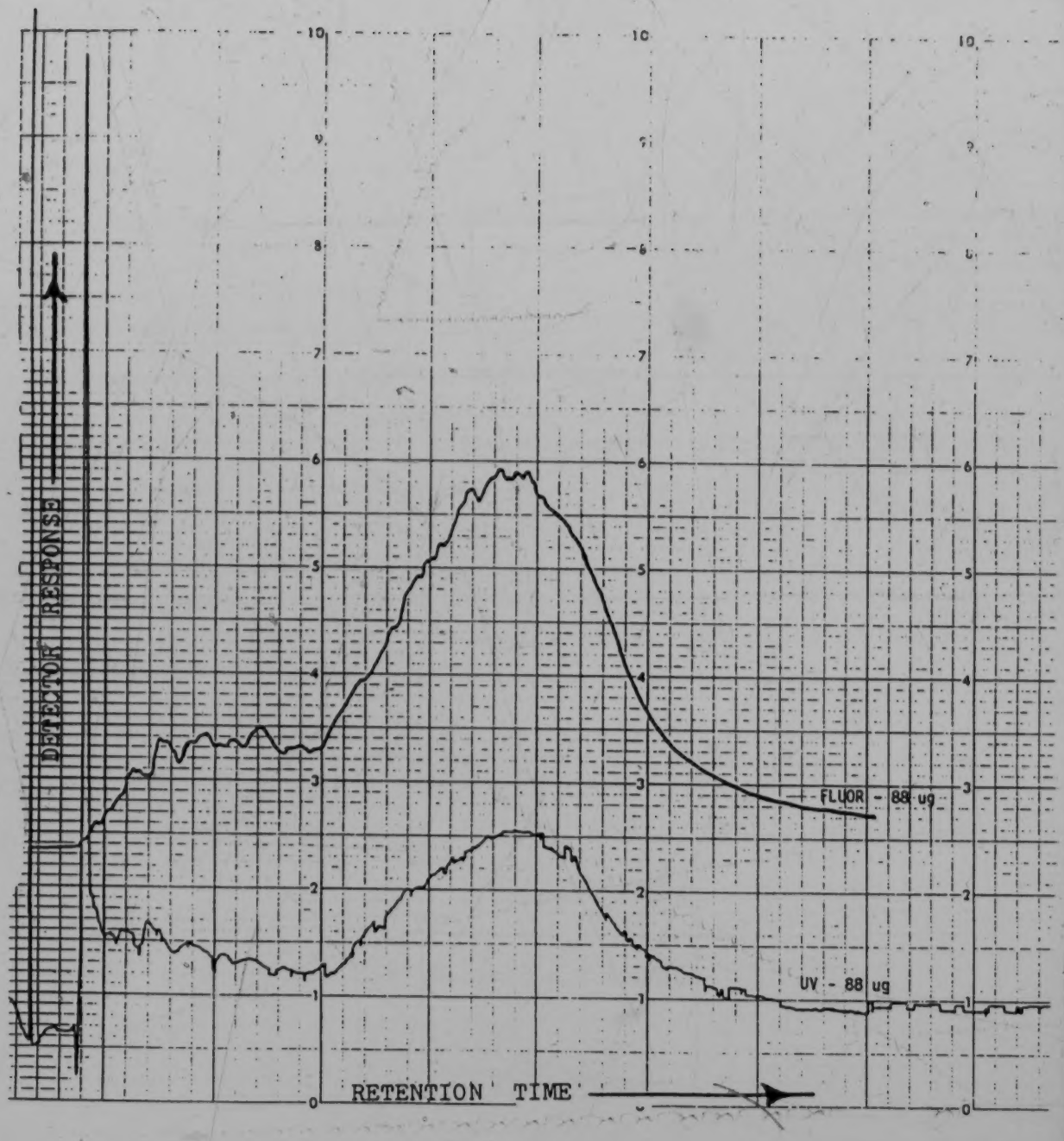


Figure 2-22 Coker Distillate Fraction A4
HPLC Analysis

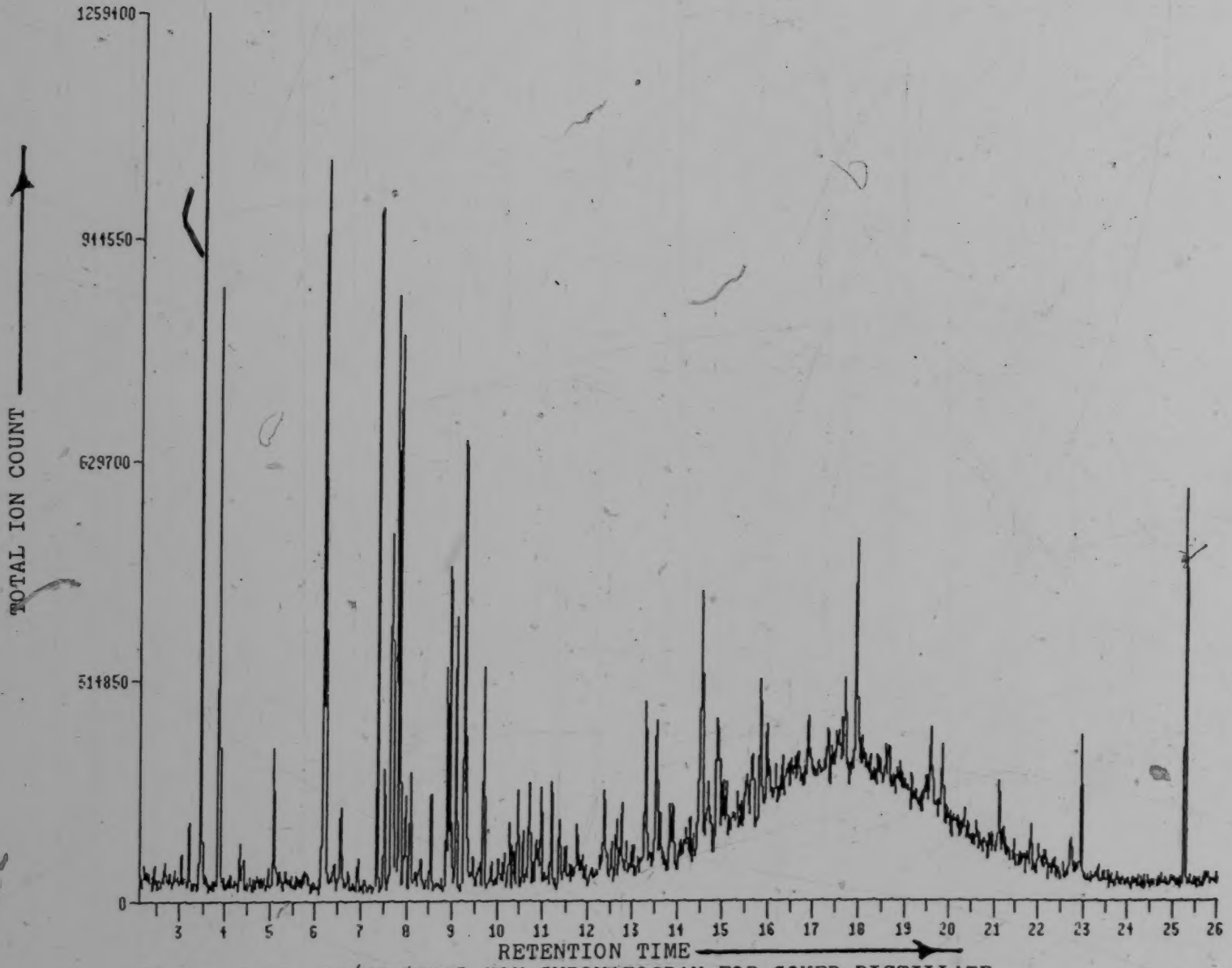
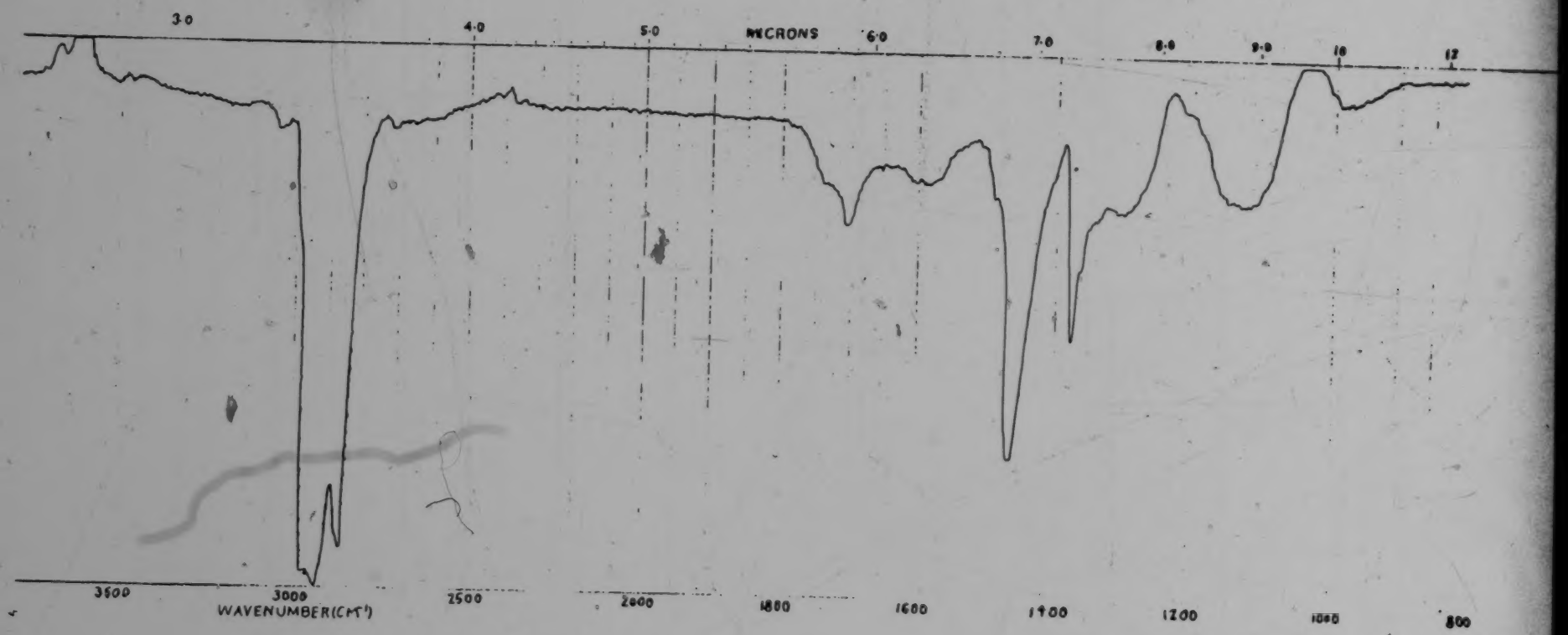


Figure 2-23 GC/MS TOTAL ION CHROMATOGRAM FOR COKER DISTILLATE ACETYLATED FRACTION A4



Solvent	CHCL3
Cell Path	0.25 mm
Reference	CHCL3
Slit	Normal
Scan Speed	Medium

Figure 2-24 DILUTED BITUMEN - ACID EXTRACT
175 mg/200 ul
INFRARED SPECTRUM

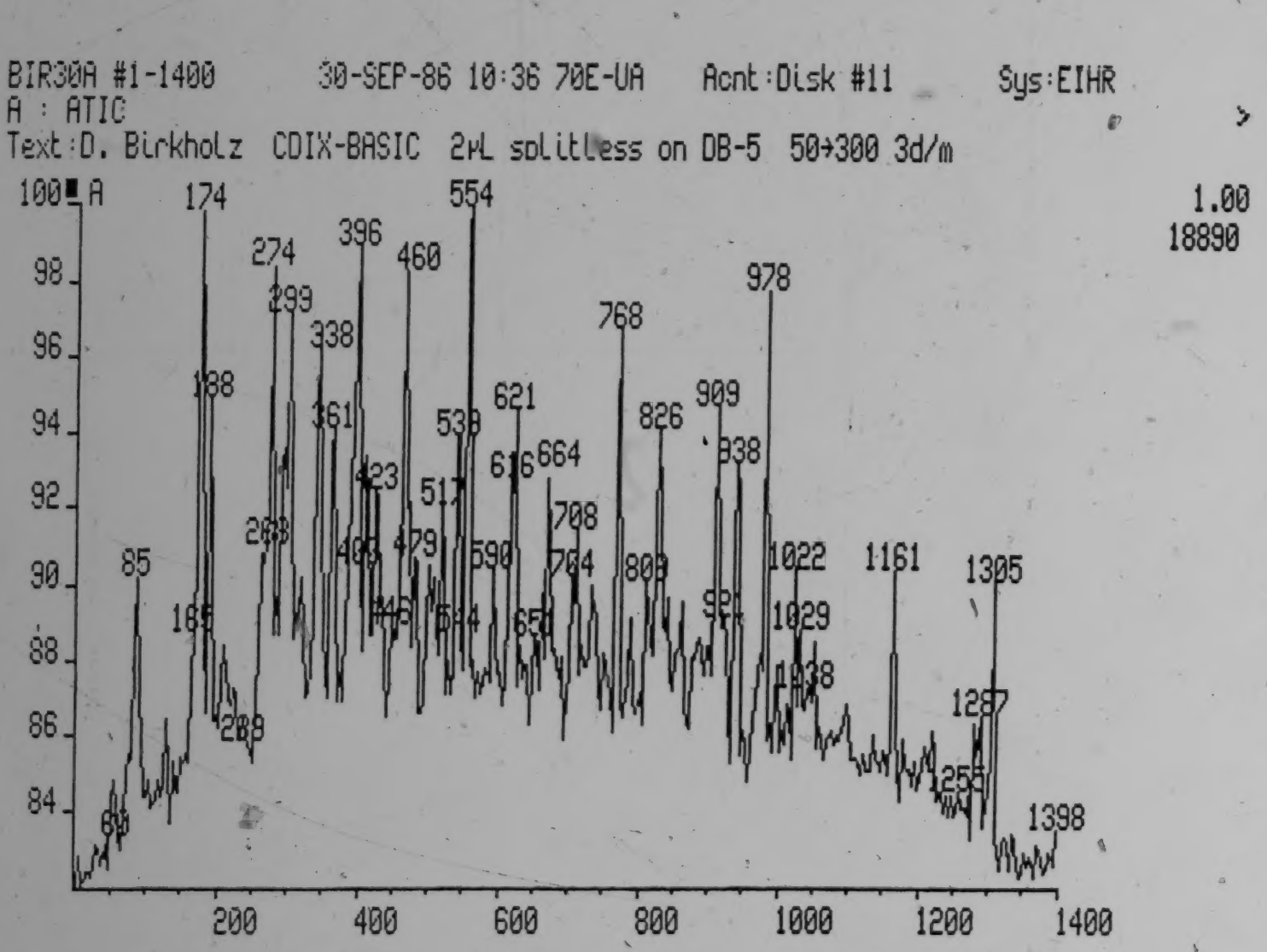
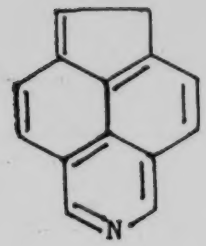
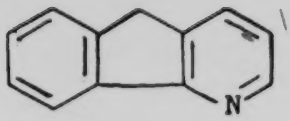


Figure 2-25 GC/HRMS TOTAL ION CHROMATOGRAM FOR COKER DISTILLATE FRACTION A3-BASIC

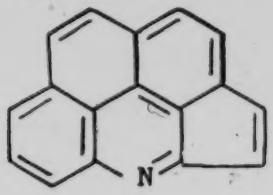
Figure 2-26 Representative Structures for Basic-PANH Identified in Coker-Distillate Fraction A3*



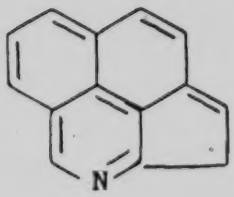
$C_{14}H_9N$ (F.W.=191)



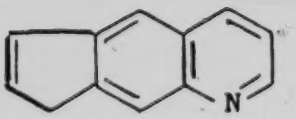
$C_{12}H_9N$ (F.W.=167)



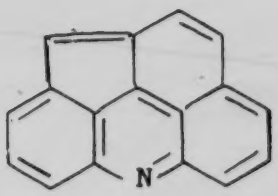
$C_{17}H_9N$ (F.W.=227)



$C_{14}H_9N$



$C_{12}H_9N$



$C_{17}H_9N$

* These are examples of many possible isomeric polyunsaturated N-containing aromatic compounds.

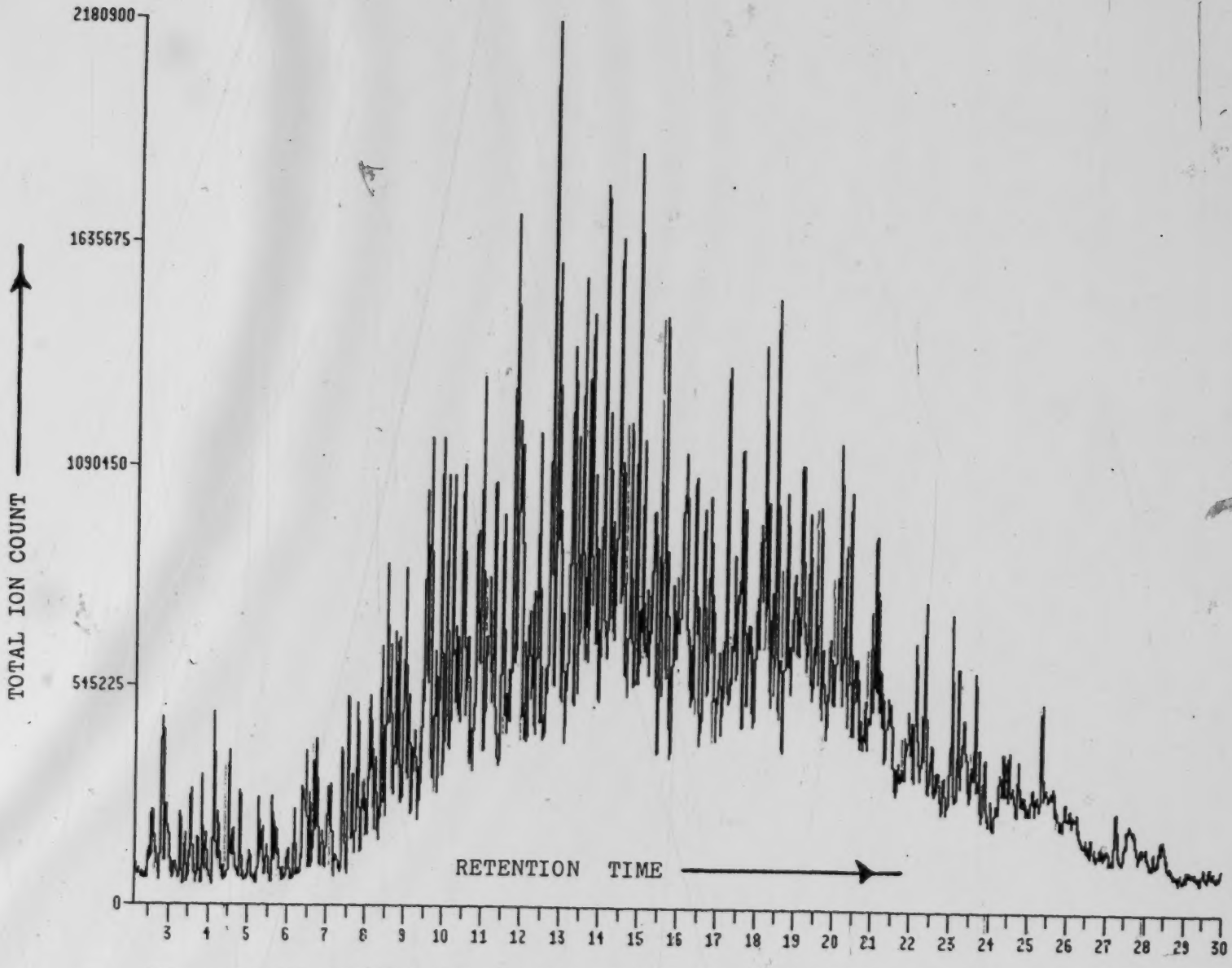


Figure 2-27 GC/LRMS TOTAL ION CHROMATOGRAM FOR COKER-DISTILLATE FRACTION A3-BASIC

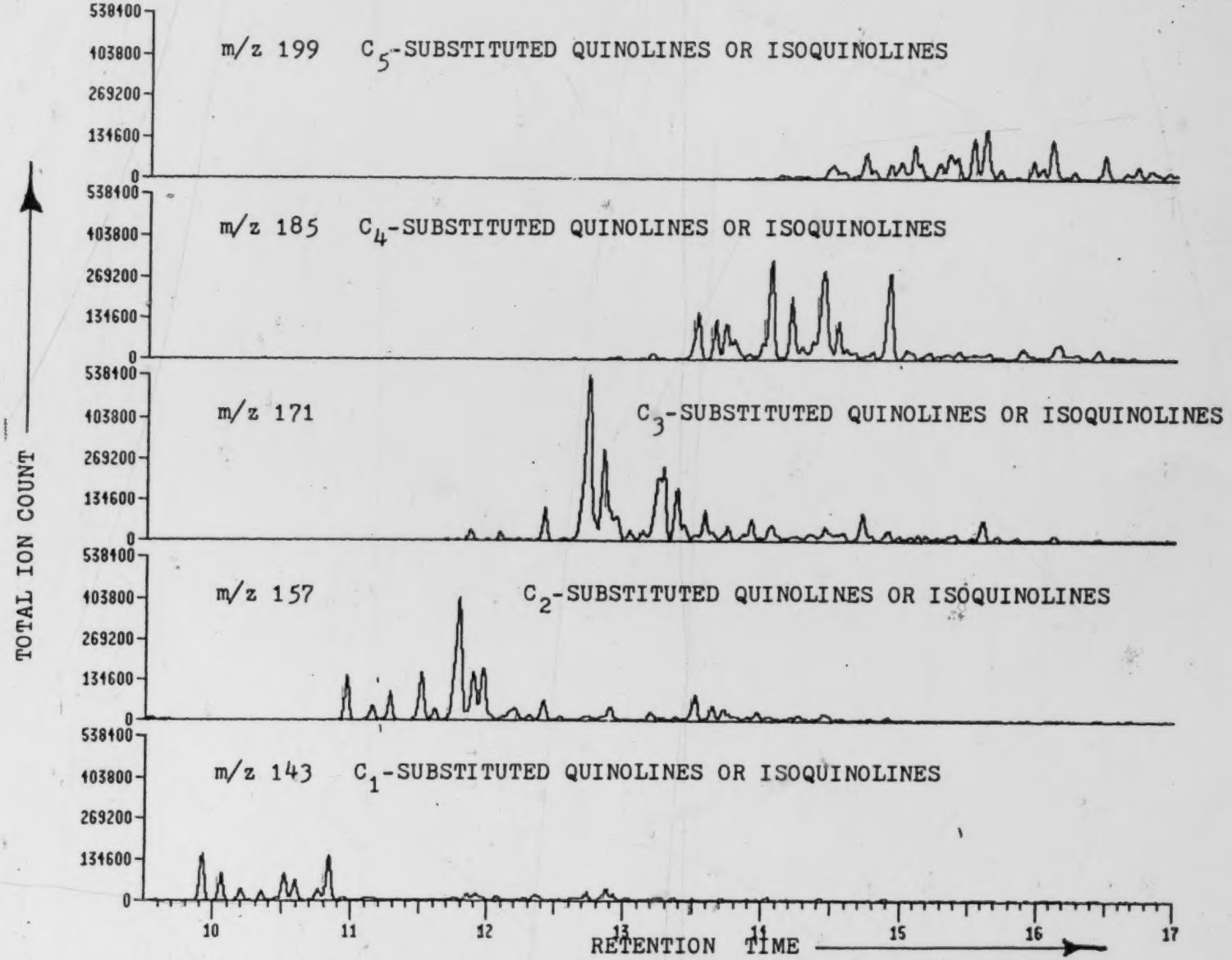


Figure 2-28 S.I.M. TRACES OF THE SUBSTITUTED QUINOLINES PRESENT IN COKER-DISTILLATE FRACTION A3-BASIC

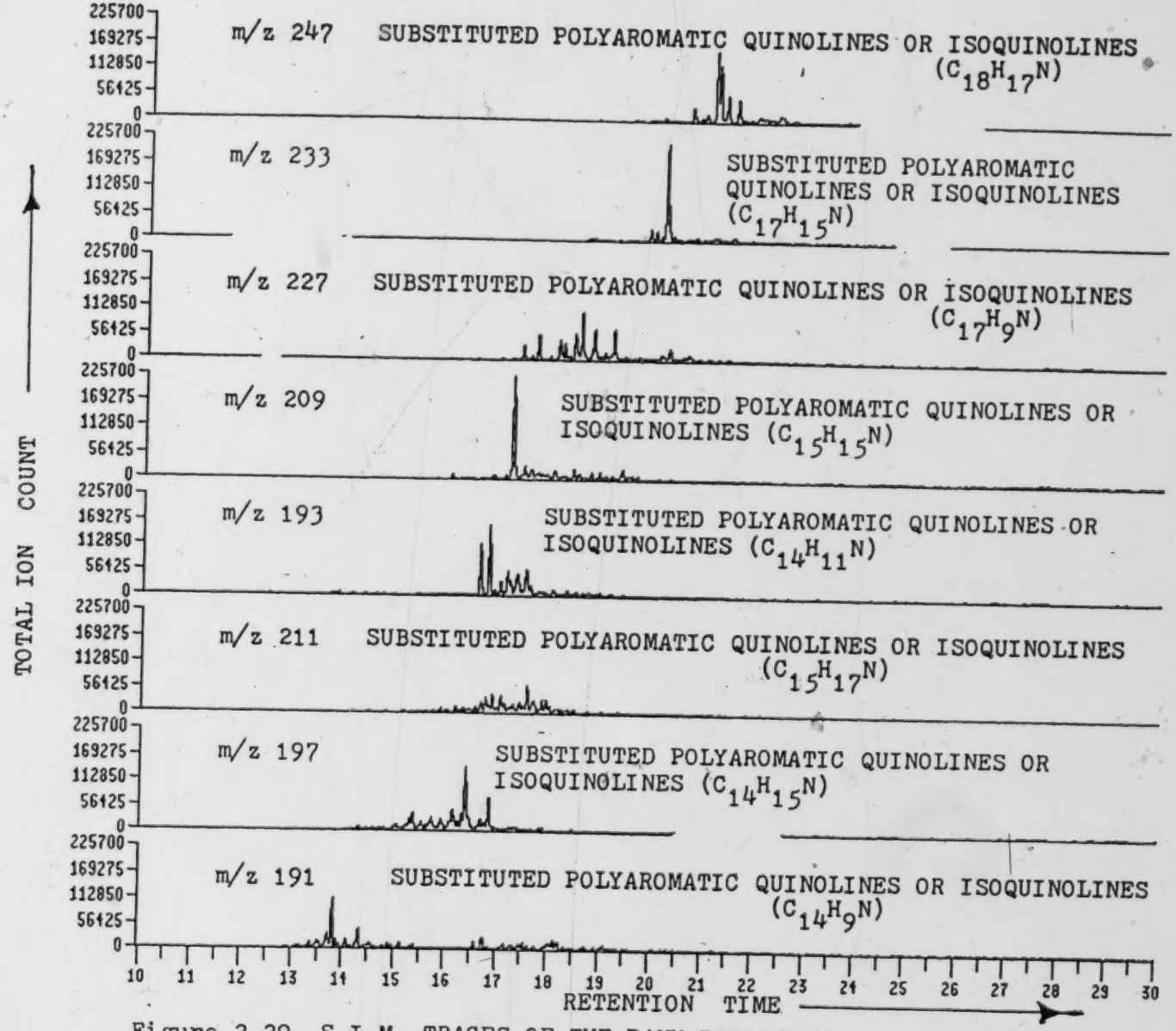

TOTAL ION COUNT
225700
169275
112850
56425
0
m/z 247 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{18}H_{17}N$)
m/z 233 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{17}H_{15}N$)
m/z 227 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{17}H_{9}N$)
m/z 209 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{15}H_{15}N$)
m/z 193 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{14}H_{11}N$)
m/z 211 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{15}H_{17}N$)
m/z 197 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{14}H_{15}N$)
m/z 191 SUBSTITUTED POLYAROMATIC QUINOLINES OR ISOQUINOLINES ($C_{14}H_{9}N$)
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
RETENTION TIME

Figure 2-29 S.I.M. TRACES OF THE PANH PRESENT IN COKER-DISTILLATE FRACTION A3-BASIC

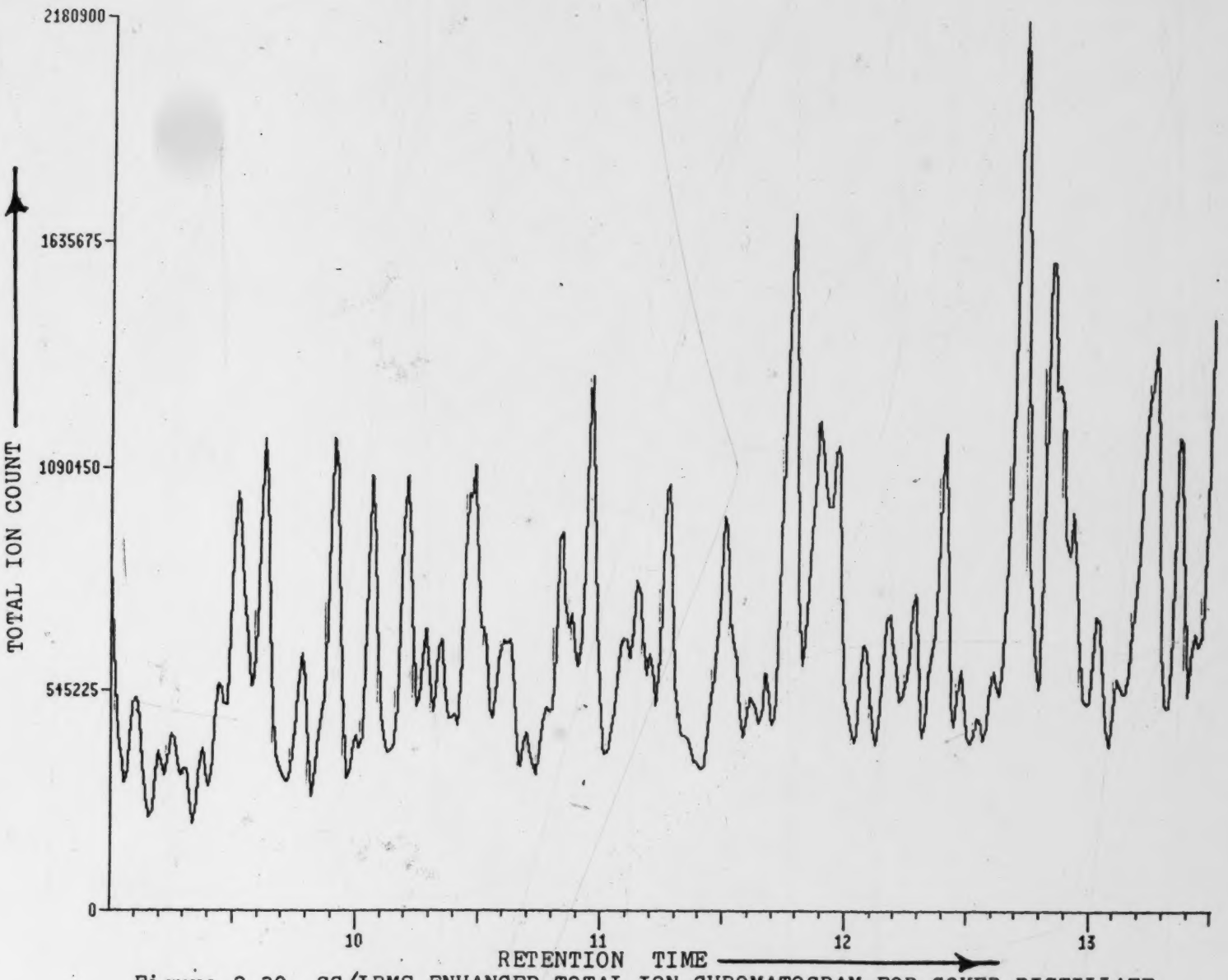


Figure 2-30 GC/LRMS ENHANCED TOTAL ION CHROMATOGRAM FOR COKER-DISTILLATE FRACTION A3-BASIC

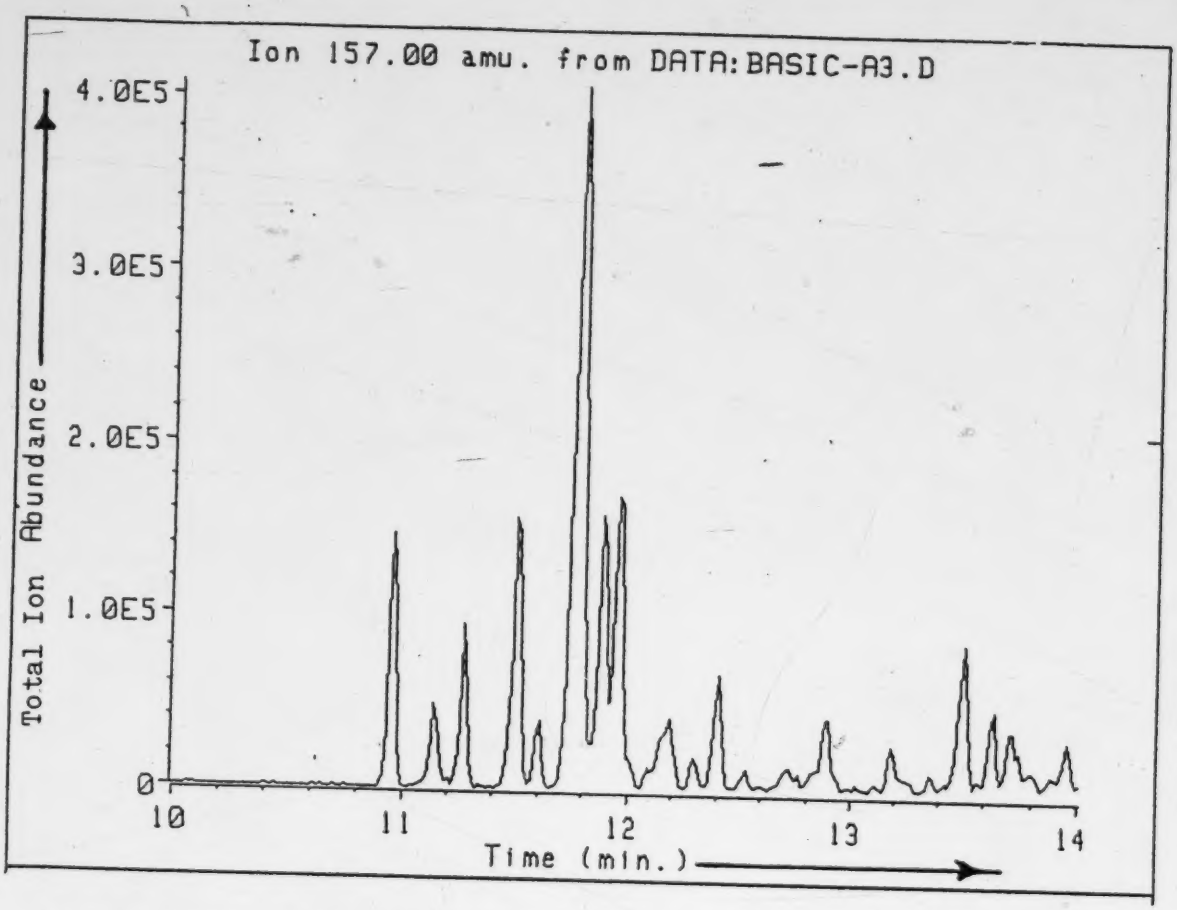


Figure 2-31 GC/LRMS ENHANCED S.I.M. CHROMATOGRAM FOR COKER-DISTILLATE FRACTION A3-BASIC

2.7 References

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3. DETERMINATION OF POLYCYLIC AROMATIC COMPOUNDS IN FISH TISSUE[1]

3.1 Introduction

Current emphasis on the development of alternate energy sources has stimulated production of synthetic fuels derived from oil shale, tar sands and coal. Although technology for producing liquid and solid fuels from these feed stocks has been available since the early 1900's (Rubin *et al.*, 1976), the chemical characterization of these products has only recently received increased attention. It is now clearly necessary to identify and control specific toxic and carcinogenic compounds in order to reduce environmental and occupational health hazards associated with the production and the combustion of these materials.

As part of a study of the uptake and elimination of toxic components isolated from thermally cracked heavy oil (coker distillate fractions) by fish, the need arose to develop a method to determine the presence of polycyclic aromatic compounds in fish tissue. Of particular interest were the polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic sulfur heterocycles (PASH), and the basic polycyclic aromatic nitrogen heterocycles (PANH). The accumulation and metabolism of toxic PAH by fish is well documented (Varanasi and Malins, 1977, Vandermuelen, 1987, Sinkkonen, 1982, and Krahn and Malins, 1982), and the accumulation of PASH from petroleum sources by fish has also been well studied (Ogata *et al.*, 1979, Ogata and Miyake,

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1 A version of this chapter has been published. Detlef A. Birkholz, Ronald T. Coutts, and Steve E. Hrudey (1988), J. Chromatogr., 449: 251 - 260.

1980, and Paasivirta *et al.*, 1981). High levels of PAH and PASH have been reported in the tissue of brown bullhead taken from the contaminated Black River in Ohio (Lee *et al.*, 1982 and Vassilaros *et al.*, 1982). Upon examination, this fish was found to have several cholangiomas (bile duct tumors) (Vassilaros *et al.*, 1982). A number of pathological conditions have been observed in fish from polluted coastal waters and estuaries. Hepatic neoplasia have been linked to the presence of aromatic hydrocarbons in bottom sediments (Malins *et al.*, 1985 and Malins *et al.*, 1984).

Recently, interest has focused on the study of PANH in environmental samples. Of particular interest are the basic PANH (which are primarily azaarenes and primary aromatic amines); these compounds are highly mutagenic as determined by the Ames test (Pelroy and Petersen, 1979, Guerin *et al.*, 1980, Wilson *et al.*, 1980, and Hsie *et al.*, 1980). Because many carcinogenic chemicals are also mutagenic (McCann *et al.*, 1975), the Ames test has been used as a screening tool in human risk assessment. The presence of basic PANH in the environment is of concern because many of these compounds are known mutagens and/or carcinogens (Dipple, 1976). For example, quinoline and all of its monomethyl isomers were found to be mutagens in the Ames *Salmonella*/microsomal assay (Dong, 1978). Recently, it has been shown that basic PANH such as acridine and quinoline are readily taken up by fish (Southworth *et al.*, 1979 and Bean *et al.*, 1985), and hepatic neoplasms and other hepatic lesions in English sole may be correlated to the presence of basic PANH in sediment (Malins *et al.*, 1985). There is ample reason then to analyze fish, taken from the environment, for

PAH, PASH and PANH in order to ascertain their bioconcentration and the effects these chemicals have on the environment.

Although many methods exist for the determination of PAH in fish, relatively few procedures exist for the determination of PASH and basic PANH in fish. Vassilaros *et al.*, (1982) presented such a method which involved alkaline hydrolysis, liquid-liquid extraction followed by alumina and gel permeation cleanup. We tried this method and found the alkaline hydrolysis method to be messy and time consuming, especially for fish with high lipid content. Furthermore, interference from biogenic compounds was observed upon analysis using GC/FID suggesting that GC was not a suitable procedure for the screening of fish samples prepared by the method of Vassilaros *et al.*, (1982).

The purpose of this research was to describe an analytical method for the extraction, cleanup and high-resolution gas chromatographic analysis of PAH, PASH and basic PANH in fish tissue.

3.2. Materials and Methods

3.2.1. Chemicals

6,7-Dimethylquinoline (6,7-DMQ) and 6,8-dimethylquinoline (6,8-DMQ) were synthesized in the University of Alberta Chemistry Department using the procedure of Manske *et al.*(1942). Purity was determined to be greater than 98 per cent using gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). Naphthalene, benzothiophene,

1-methylnaphthalene, 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, and dibenzothiophene were obtained from the Aldrich Chemical Co. and reported to be greater than 97 per cent pure. Acenaphthene-d_{10} was obtained from Merck, Sharpe and Dohme. Anhydrous sodium sulfate, Celite, concentrated hydrochloric acid, glacial acetic acid, and dichloromethane and hexane (pesticide grade; "distilled-in-glass") were obtained from Fisher Scientific. Florisil (PR grade, 60-80 mesh) was purchased from the Floridin Co.. Six normal (6N) HCl was prepared from concentrated acid and purified by extraction with methylene chloride prior to use. Anhydrous sodium sulfate and Celite were purified by continuous Soxhlet extraction with methylene chloride for 16 h. Following extraction the solvent was evaporated in a vacuum oven (maintained at 50°C) and the material stored in a convection oven maintained at 130°C until required. BioBeads SX-3 (Bio Rad) were swollen with elution solvent (methylene chloride - hexane, 1:1 v/v) overnight and wet packed into a chromatographic columnm (i.d. 19 mm) to a bed height of 50 cm. Prior to use the column was washed with several bed volumes of elution solvent. All glassware used in the analytical procedure was soaked overnight in a detergent solution (RBS-35, Pierce Chemical Co.), rinsed with hot water followed by pesticide- grade acetone and methylene chloride, and dried in an oven maintained at 250°C for 4 h.

3.2.2. Preparation and Extraction of Fish Tissue

Fish tissue samples (muscle) were prepared according to the method of Benville and Tindle (1970). This involved grinding frozen

tissue with dry ice in a Waring blender until a fine flour was obtained. Ground samples were then transferred to 250 mL wide mouth jars, covered with aluminum foil and placed in a freezer maintained at -80°C overnight in order to allow the CO_2 to sublime. Thawed subsamples (20 g) were mixed with 80 g of purified anhydrous sodium sulfate, gently packed into a glass Soxhlet extraction thimble (with extra course glass frit) containing approximately 2.5 cm of purified Celite and extracted with approximately 300 mL of methylene chloride for 6 h in a Soxhlet extractor equipped with a Freidrich condenser. Following extraction, the extract was concentrated to approximately 5 mL with the aid of a rotary evaporator operated under reduced pressure and with the water bath temperature maintained at 35°C.

3.2.3. Cleanup of Fish Tissue Extracts

Cleanup of tissue samples was performed using gel permeation chromatography. This involved diluting the extract to 10 mL with methylene chloride - hexane (1:1, v/y) and applying it to a 19 mm i.d. x 750 mm chromatographic column containing 500 mm of BioBeads SX-3 swollen with elution solvent (methylene chloride - hexane, 1:1). The column was drained to the head of the gel. The extract container was rinsed with a further 10 mL of elution solvent which was transferred to the column. Again the column was drained to the head of the gel. A 250 mL addition funnel was then filled with 230 mL of elution solvent and attached to the chromatographic column for elution. The first 75 ml of eluate (which contains primarily lipid material; but no analytes) were discarded and the next 75 mL (which contains primarily

analytes and small amounts of lipid material) collected. The eluate was then concentrated to aproximately 5 mL with the aid of a rotary evaporator.

For the analysis of PAH/PASH, hexane (20 mL) was added to the final 5 mL of the GPC eluate and the mixture was concentrated on a rotary evaporator to a volume of approximately 2 mL. This extract was applied to a chromatography column which was prepared by wet packing 10 g of 5% water deactivated Florisil (w/v) into a 10 mm i.d. chromatography column. The PAH/PASH were eluted with 50 mL of hexane, which was concentrated to 1.0 mL using a rotary evaporator followed by nitrogen blowdown.

For basic PANH such as dimethylquinolines, the 5 mL GPC eluate was quantitatively transferred to a 250 mL separatory funnel with 3 x 2 mL chloroform. An acid/base partition was performed by adding an additional 19 mL of chloroform to the separatory funnel, thoroughly mixing the contents and extracting with 3 x 25 mL 6N HCl. The combined aqueous layer was cooled in an ice bath, and basified using 6N NaOH to pH > 11. Following extraction of the basified solution with 3 x 25 mL of chloroform, the extract was dried by passage through a column containing sodium sulfate (20 g), concentrated on a rotary evaporator to approximately 2 mL and quantitatively transferred to a calibrated 5 mL centrifuge tube with 2 x 1 mL washings of chloroform. The ensuing extract was then concentrated to 1.0 mL with the aid of a nitrogen evaporator.

3.2.4. Capillary Gas Chromatography and Gas Chromatography/Mass Spectrometry

Gas chromatography was performed on a Hewlett-Packard (HP), model 5880, or a Varian, model 3500, gas chromatograph. The HP instrument was equipped with a split/splitless injector (operated in the splitless mode), autosampler, flame ionization detector, level four data processing capability and a 30 m x 0.32 mm i.d. fused silica, wall coated DB-1301 capillary column (J&W Scientific, Inc). The carrier gas was helium (linear velocity was 31 cm/sec at 280°C), and the temperature was increased from 40 to 280°C at 10°C/min beginning 1 min after injection. The oven temperature was maintained at 280°C for 20 min and the injector and detector temperatures were maintained at 270 and 300°C respectively. The injector was purged with helium 30 sec after the injection of 2 μL of sample.

The Varian GC was equipped with a split/splitless injector (operated in the splitless mode), flame ionization detector, model 600 data system and a 30 m x 0.32 mm i.d., fused silica, wall-coated DB-5 capillary column (J&W Scientific, Inc.). Conditions of analysis were the same as those employed with the HP instrument except that the linear velocity was 28 cm/sec at 300°C.

Gas chromatography/mass spectrometry was performed by interfacing a HP model 5890A GC to a HP model 5970 quadrupole mass spectrometer. The GC was equipped with a split/splitless injector (operated in the splitless mode), and a 12.5 m x 0.2 mm i.d. fused silica, wall-coated HP-1 capillary column (Hewlett-Packard). The

carrier gas was helium (linear velocity was 36 cm/sec at 300°C), and the temperature was increased from 40 to 300°C at 10°C/min beginning 1 min after injection. The oven temperature was maintained at 300°C for 8 min, and the injector, transfer line and ion source were maintained at 250, 300, and 220°C respectively. Data was acquired 2 min after injection using a HP model 59970C data system. Mass spectral scans (from 35 to 350 amu) were obtained every 1.36 seconds.

3.2.5. Recovery Study

Aliquots of prepared fish muscle (Rainbow trout; 20 g) were fortified with the following PAH/PASH: naphthalene, benzothiophene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, and dibenzothiophene. Fish muscle was also fortified with the following basic PANH: 6,7-dimethylquinoline and 6,8-dimethylquinoline. Concentrations of these chemicals ranged from 24 ng/g to 1.39 μg/g (tables 3-1 to 3-4).

3.3. Results and Discussion

The results summarized in table 3-1 were obtained from fish fortified with PAH/PASH. Analysis was performed using the Varian GC and quantitation was performed using external standards (ESTD) and an internal standard, namely acenaphthene -d_{10} (ISTD). From table 3-1 it is apparent that better than 80% recovery was obtained for the PAH/PASH at concentration levels of 0.24 - 1.1 μg/g. Precision of the method (as expressed by the percent relative standard deviation, %RSD)

was satisfactory and ranged from 4 to 10% of the mean. Little gain in precision was observed by using an internal standard during quantitation in place of external standards.

Since GC analysis using a flame ionization detector (FID) is a non-selective method of analysis, a more selective method, namely, GC/MS was used to analyze one of the fortified fish samples for PAH/PASH and the results were compared to those obtained by GC/FID. GC/MS quantitation was performed using the general principles outlined by the United States Environmental Protection Agency (1984). Results are summarized in table 3-2.

It is apparent from table 3-2 that there is close agreement between the analysis of fortified fish muscle using GC/FID and GC/MS analysis. The slightly higher recovery observed for the GC/MS analysis may be due to slight concentration of the extract during storage prior to the GC/MS analysis. The close agreement between the two methods of analysis is indicative of the excellent cleanup obtained using the GPC/Florisil combination. A chromatogram obtained from fish tissue fortified with PAH/PASH is depicted (figure 3-1). The peaks appearing before naphthalene were determined, by GC/MS, to be alkylated benzenes and were observed to be present in control fish samples. The source of these alkylated benzenes was determined to be the hexane, which was used in both the GPC and Florisil cleanup steps. The only biogenic materials found to be present in this chromatogram eluted as two significant peaks after dibenzothiophene (figure 3-1). However, these peaks were found to be present in only two samples out of 12 processed, and are of unknown origin, but were probably

introduced during GPC and/or Florisil cleanup. Rigorous calibration of the GPC and Florisil chromatography would likely eliminate these compounds.

In order to test the range of concentrations which could be detected using this method, two samples of fish tissue were fortified at low levels and subjected to GC/FID and GC/MS analysis. Table 3-3 is a summary of these findings.

From table 3-3 it can be seen that there is reasonable agreement between the GC/FID and GC/MS analysis of fish tissue samples fortified at 24 to 100 ppb. The generally higher results obtained by GC/FID are likely due to the non-selectivity of the method. Therefore, for quantitation of low levels of PAH/PASH in fish, GC/MS is the method of choice. It is worth noting that even at 20 ppb acceptable levels of recovery were observed using GC/MS analysis. GC/MS data was obtained via scanning and extracted ion current profiles were generated for the analytes of interest and internal standard prior to integration and calculation. It is anticipated that larger signal-to- noise ratios could be obtained using selected ion monitoring techniques and hence lower levels of detection could be realized. However, decreasing the analyte concentration could result in losses of material by adsorption onto glass surfaces etc., therefore a realistic detection limit of 10 - 20 ppb based on 20 g of fish and using our method is considered valid.

Table 3-4 is a summary of the results obtained for the GC/FID analysis of basic PANH in fortified fish muscle. Table 3-4 reveals excellent recoveries of dimethylquinolines from fortified fish tissue

using Soxhlet extraction followed by GPC and acid/base partition cleanup. Precision is also acceptable and similar to that observed for the PAH/PASH analyses. Furthermore, GC/MS analysis of one fortified fish sample for 6,7-dimethylquinoline produced recoveries similar to those observed using GC/FID analysis, and indicative of effective cleanup. Reference to figure 3-2 reveals a GC/FID chromatogram obtained from fortified fish muscle and virtually free of any biogenic interfering material.

We have developed an analytical method capable of detecting accurate and precise levels of PAH/PASH and basic PANH in samples of fish muscle. Good agreement was observed between GC/FID and GC/MS analysis which indicates substantial removal of biogenic material during cleanup. The use of GC/FID as a screening method is desirable because of accessability by most laboratories and low cost relative to GC/MS. This method was applied to the analysis of muscle obtained from fish exposed to 6,7-dimethylquinoline, 6,8-dimethylquinoline and benzothiophene. Concentrations in muscle were determined immediately after exposure and after depuration. A summary of concentration in muscle observed in fish after exposure to the three chemicals is shown in table 3-5. From this table it is apparent that PANH and PASH are bioconcentrated by fish from water.

A summary of concentrations in muscle observed in fish following depuration of the three chemicals is given in table 3-6. From table 3-5 and 3-6 it is apparent that PANH and PASH have different rates of uptake and elimination. The uptake, elimination, and biotransformation of PANH by fish is more fully described by Birkholz *et al.* (1988). The

uptake, elimination and biotransformation of PASH is described by Dromey (1988).

In conclusion we have developed a precise and accurate method for the determination of PAH, PASH and PANH in fish muscle. The sensitivity of the method was found to be more than adequate when applied to study of the uptake and elimination of PASH and PANH by exposed fish.

3.4. Tables

Table 3-1 Recovery of Polycyclic Aromatic Hydrocarbons/Polycyclic Aromatic Sulfur Heterocycles (PAH/PASH) From Fortified Fish Muscle

Analyte	Conc. (μg/g)	No. Rep. (n)	Mean Recov. (%)	RSD[2] (%)	Quant. Method (ESTD[3]/ ISTD[4])
Naphthlalene	0.97	4	86	5.1	ESTD
	0.97	4	85	4.8	ISTD
Benzothiophene	1.1	4	85	4.5	ESTD
	1.1	4	81	5.7	ISTD
1-Methylnaphthalene	1.0	4	88	4.3	ESTD
	1.0	4	85	5.4	ISTD
2,6-Dimethylnaphthalene	1.0	4	88	6.1	ESTD
	1.0	4	87	6.6	ISTD
2,3,5-Trimethylnaphthalene	0.24	4	93	10.1	ESTD
	0.24	4	85	9.0	ISTD
Dibenzothiophene	1.0	4	92	9.2	ESTD
	1.0	4	84	6.4	ISTD

2 RSD%. relative standard deviation in percent

3 ESTD: external standard method of quantitation

4 ISTD: internal standard method of quantitation

Table 3-2 Analysis of Fortified Fish Muscle by GC/FID and GC/MS

Analyte	Conc. (μg/g)	Percent Recovery (GC)	Percent Recovery (GC/MS)
Naphthalene	0.97	84	89
Benzothiophene	1.1	81	93
1-Methylnaphthalene	1.1	88	91
2,6-Dimethylnaphthalene	1.0	90	100
2,3,5-Trimethylnaphthalene	0.24	92	95
Dibenzothiophene	1.0	88	96

Table 3-3 GC/FID and GC/MS Analysis of Fish Tissue Fortified at 0.1 μg/g

Analyte	Conc. (ng/g)	Per Cent Recovery			
		Spike 1		Spike 2	
		GC/FID	GC/MS	GC/FID	GC/MS
Naphthalene	96.9	114	93	256	138
Benzothiophene	104	107	68	153	65
1-Methylnaphthalene	106	93	79	86	53
2,6-Dimethylnaphthalene	100	81	77	65	68
2,3,5-Trimethylnaphthalene	24		74	155	65
Dibenzothiophene	104	88	77	82	78

Table 3-4 Analysis of Dimethylquinolines in Fortified Fish Muscle

Analyte	Conc. (μg/g)	(n)	Mean Recovery (%)	RSD (%)
6,8-Dimethylquinoline	1.39	4	96	8.2
6,7-Dimethylquinoline	1.15	4	98	7.0

Table 3-5 Muscle Concentration Observed in Fish Exposed to PANH[1] and PASH[1]

Analyte	Mean Exposure Conc. (ug/mL)	Exposure Time (h)	Mean Muscle Conc. (μg/g)	Number of Fish Exposed
6,7-dimethylquinoline[2]	1.0	7.5	6.1	3
6,8-dimethylquinoline[2]	1.1	8.0	14	3
Benzothiophene[3]	0.67	8.0	19	3

1 Abbreviations - PANH: polycyclic aromatic nitrogen heterocycles, and PASH: polycyclic aromatic sulfur heterocycles

2 Data taken from Birkholz et al., 1988

3 Data obtained from Dromey, 1988

Table 3-6 Concentration of PANH[1] and PASH[1] in Fish Muscle Following Exposure and Depuration

Analyte	Number of Exposures	Mean Exposure Conc. (mg/L)	Exposure Time (h)	Depuration Time (h)	Muscle Conc. (μg/g)
6,7-dimethylquinoline[2]	3	0.97	9.5	69	0.56
6,8-dimethylquinoline[2]	3	1.1	7.0	63	0.49
benzothiophene[3]	3	0.67	8.0	65	2.2

1 Abbreviations - PANH: polycyclic aromatic nitrogen heterocycles and PASH: polycyclic aromatic sulfur heterocycles

2 Data obtained from Birkholz et al., 1988

3 Data obtained from Dromey, 1988

3.5. FIGURES

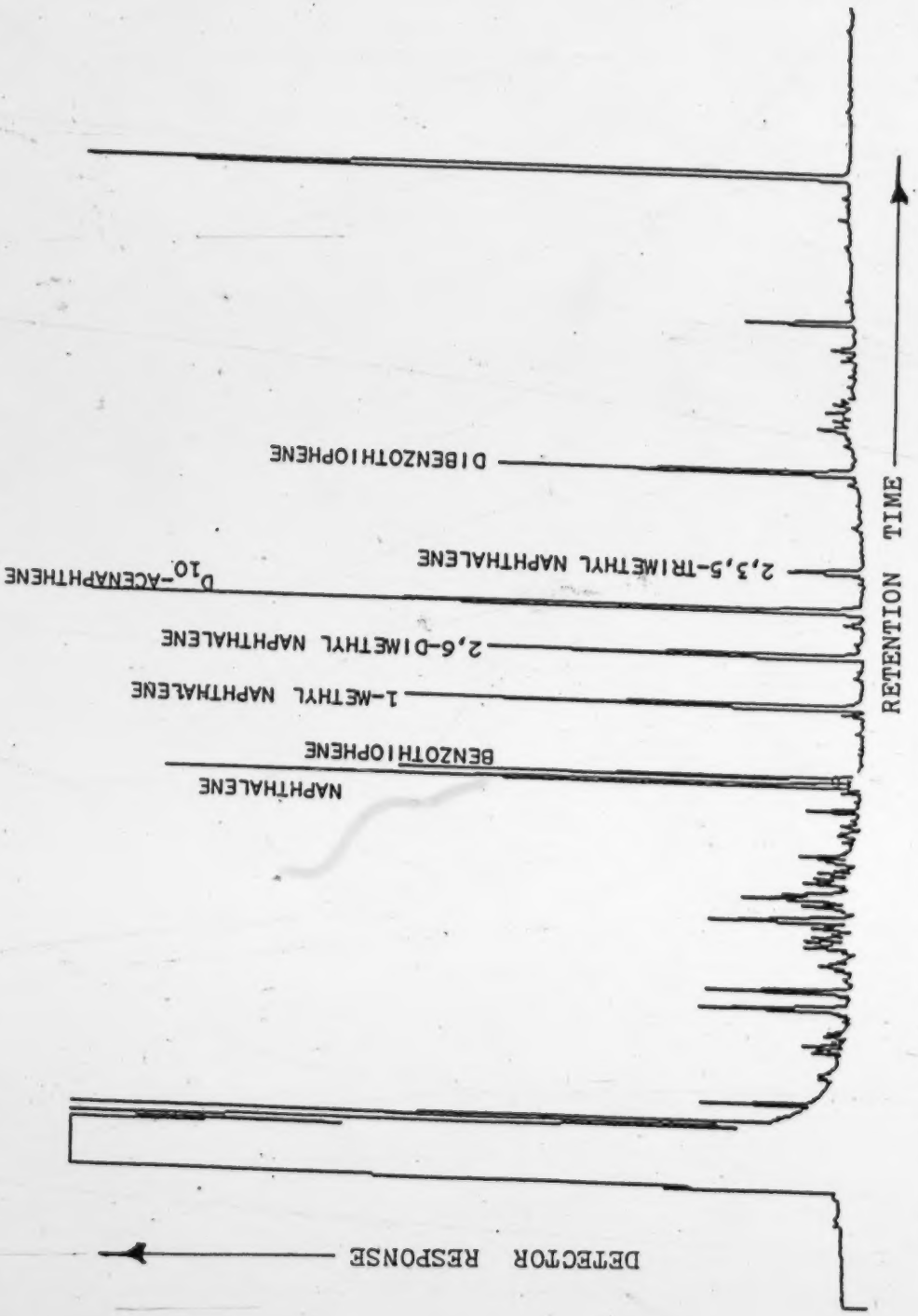

Figure 3-1 FISH MUSCLE FORTIFIED WITH PAH/PASH

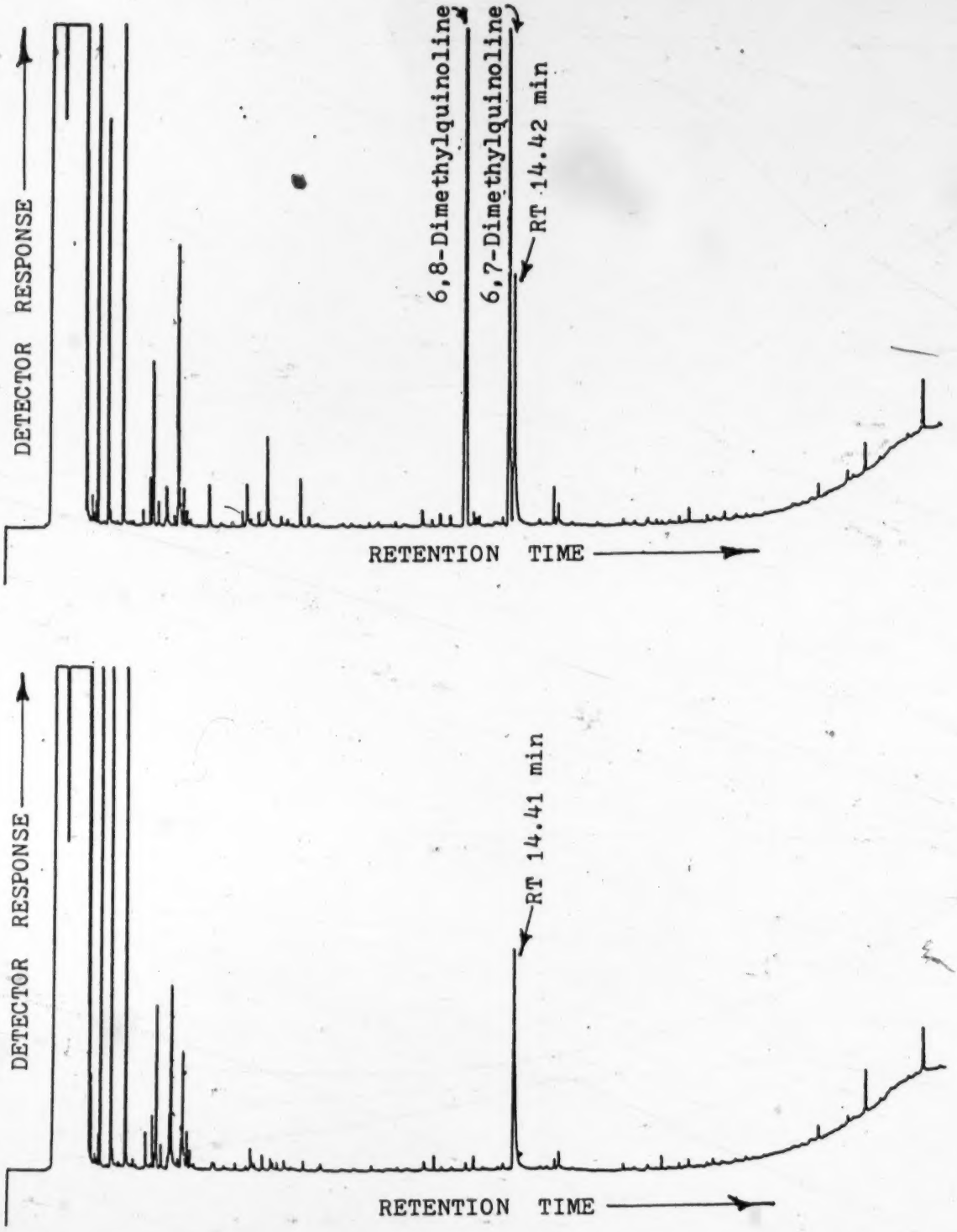

Figure 3-2

UPPER - FISH MUSCLE FORTIFIED WITH 6,7 AND 6,8-DIMETHYL QUINOLINE
LOWER - CONTROL FISH MUSCLE

3.6. References

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4. UPTAKE AND BIOTRANSFORMATION OF 6,7-DIMETHYLQUINOLINE AND 6,8-DIMETHYLQUINOLINE BY RAINBOW TROUT (*Salmo gairdneri*)

4.1. Introduction

The occurrence of polycylic aromatic hydrocarbons (PAH) in the environment has been widely studied. Much less attention has been focused on the types and amounts of heterocyclic analoques of PAH, particularly polycylic aromatic nitrogen heterocylces (PANH). Since many of the sources of PANH and PAH are the same (e.g., combustion processes and petroleum products), these heterocycles would be expected to be widespread in areas of concentrated human activities. A survey of the literature indicates that PANH such as quinolines and benzoquinolines may be widespread in the environment. They have been identified in air particulate matter from Europe (Brocco *et al.*,1973), the United States (Dong *et al.*,1977), and the southern North Atlantic Ocean (Hahn, 1980). Moreover, recent data indicate that these compounds are also present in ambient air in the vapor phase and at higher levels than previously reported on particulate matter (Adams *et al.*,1982). PANH have also been found in lake and marine sediments (Furlong and Carpenter, 1982; Wakeham, 1979; and Blumer *et al.*, 1977) and groundwater adjacent to an underground coal gasification site (Stuermer *et al.*, 1982). The presence of these compounds in the environment has been mainly attributed to the use of fossil fuels (Furlong and Carpenter, 1982; and Wakeham, 1979). The occurrence of PANH in coal tar has been known since the early 1800s (Acheson, 1967).

They have been identified more recently in both natural and synthetic crudes (Ford *et al.*,1981; Tomkins and Ho, 1982; and Schmitter *et al.*, 1982) and subsequently derived distillates and oils (McKay *et al.*, 1976; Novotny *et al.*, 1980 and Later *et al.*, 1981). In petroleum oils, the total fraction of basic organic nitrogen has been estimated to range from 0.2 to 0.5% (Aksenov *et al.*, 1979); however, for coal-derived liquids this range may approach 10-20% (Paudler and Cheplen, 1979 and Later *et al.*, 1981).

The presence of PANH in the environment is of concern because many of these compounds have been reported to be toxic (Sidhu and Blair, 1975 and Schultz *et al.*, 1982), teratogenic (Dumont *et al.*,1979), mutagenic, and/or carcinogenic (Ho *et al.*, 1979; Hirao *et al.*, 1976; and Dipple, 1976). For example, quinoline and all of its monomethyl isomers were found to be mutagens in the Ames *Salmonella* assay (Dong *et al.*, 1978). It has also been shown that quinoline, 4-methylquinoline, and 8-methylquinoline initiated skin tumors in SENCAR mice (LaVoie *et al.*, 1984).

The uptake, elimination, and metabolism of PANH (or basic azaarenes) by fish has received little study. Considering the many toxic, mutagenic, teratogenic and carcinogenic effects that have been demonstrated for these compounds, it is important that studies of the origin, concentration, fate, and effects of these compounds be conducted.

We report on the uptake, elimination, and metabolism of two dimethylquinolines namely 6,7- and 6,8-dimethylquinoline in rainbow trout, (*Salmo gairdneri*).

4.2. Experimental

4.2.1 Chemicals

6,7-Dimethylquinoline (6,7-DMQ) and 6,8-dimethylquinoline (6,8-DMQ) were synthesized in the University of Alberta Chemistry Department using the procedure of Manske *et al.* (1942). Purity was determined to be greater than 98 per cent using gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). Pentafluorobenzoyl chloride, 6-methylquinoline, 8-methylquinoline, 1-hydroxymethylnaphthalene and 8-hydroxyquinoline were obtained from Aldrich and reported to be greater than 97 per cent pure. Benzyl alcohol (98%), acetic anhydride (97%), Celite, anhydrous sodium sulfate, phosphoric acid (85%), ammonium hydroxide (28 - 30%) and pesticide grade solvents (chloroform, methylene chloride and methanol) were obtained from Fisher Scientific. α–Naphthyl-ß-D-glucuronic acid and glucuronidase (type L-11 from *P. vulgata*) were obtained from Sigma Chemical Company. The ß-glucuronidase possessed sulfatase activity. Organic-free water was obtained by passing distilled water through a Norganic trace organic removal cartridge (Millipore Corp.). Ammonium phosphate buffer (pH 6.5) was prepared by adding 1.0 mL of phosphoric acid to 1 L of organic free water and titrating to pH 6.5 by dropwise addition of ammonium hydroxide (28 - 30%). Potassium phosphate buffer was prepared by titrating 50 mL of 0.1 M potassium dihydrogen orthophosphate (Fisher Scientific) with 0.1 M sodium hydroxide (Fisher) to pH 7.0. Acetate buffer (0.2 M) was prepared by titrating 50 mL of 0.2 M acetic

acid with 0.2 M sodium acetate to pH 5. Acetate buffer (2.0 M) was prepared by titrating 50 mL of 2.0 M acetic acid with 2.0 M sodium acetate to pH 5.

ß-Glucuronidase solution (Sigma, G-8132) was prepared by dissolving 10 - 12 mg of the enzyme in 500 µL of 0.2 M acetate buffer. One hundred microliters of this solution gave approximately 2000 units of enzymatic activity.

Anhydrous sodium sulfate and Celite were purified by Soxhlet extraction with methylene chloride for 16 h. Following extraction the solvent was evaporated in a vacuum oven (maintained at 50°C) and the material stored in a convection oven maintained at 130°C until required.

4.2.2. Fish Exposures

Rainbow trout used in tissue distribution and metabolism studies averaged 255 ± 22 mm in length and weighed 217 ± 52 g (n=15). For exposures, three fish were held without food, each in a separate glass aquarium that contained 50 L of charcoal-filtered tap water. Tests were conducted under static conditions of 12 - 17 °C with a light:dark cycle of 16:8 h. Aquaria were not aerated, and water was exchanged at 7 and 9.5 h for fish that were depurating 6,8-DMQ and 6,7-DMQ respectively. Fish were exposed to 6,7-DMQ and 6,8-DMQ in aquarium water, removed from each of three replicate aquaria: sacrificed by a blow to the head at two time intervals: 7.5 and 8 h after exposure to

6,7-DMQ and 6,8-DMQ respectively, and 69 and 63 h after depuration; then dissected. A further exposure (7.5 h) and depuration (96 h) was conducted using 6,7-DMQ because dissection of one of the previous fish failed to generate a bile sample for analysis.

One fish was also exposed to 6-methylquinoline for 7.5 h and sacrificed. The bile was removed for chemical analysis. Another fish was similarly exposed to 8-methylquinoline for 7.5 h, sacrificed and the bile removed for chemical analysis. Exposure concentrations were calculated to be 1 mg/L although no exact measurements were taken. The purpose of these two exposures was to generate data that might aid in the identification of metabolites obtained during the exposure of fish to 6,7-DMQ and 6,8-DMQ.

4.2.3. Quantitative Analysis for Dimethylquinolines

Samples of bile, liver, muscle and carcass (remaining tissue) were obtained from exposed and control fish and analyzed for 6,7-DMQ and 6,8-DMQ. All tissues were weighed (wet weight) immediately after dissection, vacuum packed in freezer bags, and frozen at -80°C prior to processing and analysis. Water samples (n=6-9), for the analysis of 6,7-DMQ and 6,8-DMQ, were taken from each of the exposure tanks using 3.5 mL septum-cap vials.

Analysis of 6,7-DMQ and 6,8-DMQ in water used for trout exposure was performed by reverse phase liquid chromatography. Fish tissue samples (muscle and carcass) were prepared according to the method of Benville and Tindle (1970). This involved grinding the frozen tissue

with dry ice in a Waring blender until a fine flour was obtained. Ground samples were then transferred to 250 mL wide mouth jars, covered with aluminum foil and place in a freezer maintained at -80°C overnight in order to allow the CO_2 to sublime. Thawed subsamples (20 g) were mixed with 80 g of purified anhydrous sodium sulfate, gently packed into a glass Soxhlet extraction thimble containing approximately 1 inch of purified Celite and extracted with methylene chloride for 6 h in a Soxhlet extractor. Following extraction, the extract was concentrated to approximately 5 mL with the aid of a rotary evaporator.

Cleanup of tissue samples (muscle and carcass) was performed using gel permeation chromatography and an acid/base partitioning step. This involved diluting the extract to 10 mL with methylene chloride:hexane (1:1) and applying it to a 19 mm i.d. x 750 mm chromatographic column containing 500 mm of Bio-Beads SX-3 swollen with elution solvent (methylene chloride:hexane, 1:1). After draining the column to the head of the gel, a further 10 mL of elution solvent was transferred to the column after rinsing the extract container. Again the column was drained to the head of the gel. A 250 mL addition funnel was then filled with 230 mL of elution solvent and attached to the chromatographic column for elution. The first 75 mL of eluate were discarded and the next 50 mL collected. The eluate was then concentrated to approximately 5 mL with the aid of a rotary evaporator and quantitatively transferred to a 250 mL separatory funnel with 3 x 2 mL chloroform.

An acid/base partition was performed by adding an additional 19

mL of chloroform to the separatory funnel, thoroughly mixing the contents and extracting with 3 x 25 mL 6N HCl. The upper HCl layer was removed, collected in a 250 mL Erlenmeyer flask, cooled in an ice bath, and basified using 6N NaOH to pH > 10. Following extraction of the basified solution with 3 x 25 mL of chloroform, the extract was dried by passage through a 20 g sodium sulfate column, concentrated on a rotary evaporator to approximately 2 mL and quantitatively transferred to a calibrated 5 mL centrifuge tube with 2 x 1 mL washings of chloroform. The ensuing extract was then concentrated to 1.0 mL with the aid of a nitrogen evaporator.

All cleaned-up tissue extracts (muscle and carcass) were analyzed for 6,7-DMQ and 6,8-DMQ by gas chromatography/flame ionization detection (GC/FID).

Liver samples were analyzed for 6,7-DMQ and 6,8-DMQ using a modification of the method of Varanasi *et al.* (1982). The modification involved the use of potassium phosphate buffer (pH 7.0) instead of saline. Liver (1-3 g) was transferred to a 50 mL centrifuge tube and 4 mL of potassium phosphate buffer (pH 7.0) was added. After homogenizing with a polytron for approximately 1 min the probe was washed with 5 x 2 mL methanol and the washings collected in the centrifuge tube. After the addition of 5 mL chloroform, the mixture was vortexed for approximately 1 min, an additional 5 mL of chloroform was added and the mixture vortexed again for 1 min. At this point, 5 mL of organic-free water was added, the mixture vortexed and centrifuged at 2000 rpm for 5 min. The lower chloroform/methanol layer was removed by pipette, dried through a 10 g sodium sulfate column and

collected in a 250 mL round bottom flask. Following the extraction of the aqueous/solids portion with an additional 10 mL chloroform, the mixture was again centrifuged and the lower chloroform layer removed, dried through the sodium sulfate column and collected in the round bottom flask. The extract was then concentrated to 1.0 mL with the aid of a rotary evaporator, calibrated 5 mL centrifuge tube and nitrogen evaporator. The aqueous portion of the sample was saved for further analysis.

4.2.4. Enzymatic Hydrolysis and Isolation of Metabolites from Conjugates

Bile samples were analyzed for 6,7-DMQ and 6,8-DMQ as well as conjugated metabolites using the method of Baird *et al.* (1977). Aliquots of bile (200 μL) were weighed into a 15 ml centrifuge tube, diluted to 1 mL with 0.2 M acetate buffer and incubated with 2000 units of ß-glucuronidase for 2 h at 37^{o}C in a shaking incubator. After enzyme treatment, the solutions were extracted using the following regime. Two and one-half mL of methanol and 1.25 mL chloroform were added to the enzymatically hydrolyzed sample and vortexed for 1 min. A further 1.25 mL of chloroform was added, followed by vortexing for 1 min and the addition of 1.25 mL of organic free water. After vortexing for an additional minute the lower chloroform/methanol layer was removed with a Pasteur pipette, dried by passage through a 1 g sodium sulfate column, and collected in a 5 mL calibrated centrifuge tube. An additional extraction was performed with 2 mL chloroform and the

extract dried and collected as previously described. The combined extracts were then concentrated to 1.0 mL with the aid of a nitrogen evaporator.

Isolation of conjugated metabolites from liver followed the procedure of Varanasi *et al.* (1982). The aqueous-methanol phase of the liver extraction (with protein removed by centrifugation) was evaporated to 6 mL with a nitrogen evaporator and made 0.2 M with acetate buffer (2.0 M). This mixture was incubated with 2000 units of ß-glucuronidase for 2 h at 37°C. After enzyme treatment the solution was extracted serially by vortexing with 10 mL methanol, 2 x 5 mL chloroform, and 5 mL organic free water. The lower chloroform-methanol layer was removed by pipette, dried through a 10 g sodium sulfate column, and collected in a 250 mL round bottom flask. Following a further extraction with 10 mL chloroform, the extract was again dried and the combined extractions were concentrated to 1.0 mL with the aid of a rotary evaporator, 5 mL calibrated centrifuge tube and a nitrogen evaporator.

All liver and bile extracts were analyzed for 6,7-DMQ, 6,8-DMQ and hydrolyzed conjugated metabolites using GC/FID and gas chromatography/nitrogen-phosphorus detection (GC/NPD). Parent compound and compounds released from conjugated metabolites obtained from the livers and bile of fish exposed to 6,7-DMQ were quantitated using 6,7-DMQ and 1-hydroxymethylnaphthalene as reference standards. Hydrolyzed conjugated metabolites and parent compound observed to be present in the liver and bile of fish exposed to 6,8-DMQ were quantitated using 6,8-DMQ and 8-hydroxyquinoline as reference

standards.

4.2.5. Quality Control/Quality Assurance

Aliquots of tissue (muscle, carcass and liver) were obtained from control fish and analyzed before and after fortification with 6,7-DMQ and 6,8-DMQ. The efficiency of the enzymatic hydrolysis was tested by hydrolyzing replicate aqueous solutions of α-naphthyl-ß-D-glucuronic acid (200 μg/mL) and extracting and analyzing the hydrolyzate for 1-naphthol by GC/FID.

4.2.6. Acetylation

Acetylation of enzymatically hydrolyzed and extracted bile samples as well as of benzyl alcohol followed the procedure of Hargesheimer *et al.* (1981). One mL of the chemical standard (in methanol) containing approximately 200 μg of chemical was added to a 50 mL centrifuge tube. Water (4 mL) was added along with 250 μL of acetic anhydride and the mixture vortexed. Solid sodium bicarbonate (Fisher Scientific) was then added and mixing continued until the evolution of CO_2 ceased and an excess of sodium bicarbonate was apparent. Following extraction with 3 x 1 mL of chloroform, the combined extract was dried by passage through a 1 g sodium sulfate column and collected in a 5 mL calibrated centrifuge tube. Volume was adjusted to 5.0 mL, with chloroform, prior to GC/FID and GC/MS analysis.

Enzymatically hydrolyzed and extracted bile samples obtained from fish exposed to 6,7-DMQ and 6,8-DMQ were acetylated similarly except that 0.5 mL of extract was first exchanged into methanol. This was accomplished by diluting the extract to 3.5 mL with methanol and reconcentrating to 0.5 mL with the aid of a nitrogen evaporator. Because chloroform and methanol form an azeotrope which is 87% chloroform, this procedure offers an effective and efficient way to exchange the extract while at the same time minimizing loss of volatile material which might be lost if the extract were taken to dryness prior to exchange. Following the exchange, acetylation was performed as described above except that the final extract was concentrated to 0.5 mL before GC/FID and GC/MS analysis.

4.2.7. Pentafluorobenzoylation

Acylation of enzymatically hydrolyzed and extracted bile samples as well as benzyl alcohol was performed using the method of Coutts *et al.* (1987). Two mL benzene containing approximately 100 μg of benzyl alcohol was added to a 15 mL screw cap test tube and vortexed with 10 μL of pentafluorobenzoyl chloride. After addition of 2 ml of saturated sodium bicarbonate solution and 2 ml of benzene, and attachment of a Teflon lined screw cap the vial was mechanically shaken for 20 min. The upper benzene layer was removed, dried by passage through a 1 g sodium sulfate column and concentrated to 1 mL using a nitrogen evaporator prior to GC/FID and GC/MS analysis. Enzymatically hydrolyzed and extracted bile samples (0.5 mL) were diluted to 2.5 mL

with benzene and acylated as above. The resulting extract was concentrated to 0.5 mL using a nitrogen evaporator prior to GC/FID and GC/MS analysis.

4.2.8. Gas Chromatography and Mass Spectrometry

Gas chromatography/flame ionization analyses were performed using a Hewlett-Packard (HP, model 5880), and a Varian (model 3500) gas chromatograph. The HP instrument was equipped with a 30 m x 0.32 mm i.d. fused silica, wall-coated DB-1301 capillary column (0.25 μm film thickness, J&W Scientific). The carrier gas was helium (linear velocity was 31 cm/sec at 280^{o}C), and the temperature was increased from 40 to 280^{o}C at 10^{o}C/min beginning 1 min after injection. The oven temperature was maintained at 280^{o}C for 20 min and the injector and detector were maintained at 270 and 300^{o}C respectively. Portions (2 uL) of the concentrated extract containing nonconjugated metabolites were injected into the capillary injector with the purge valve closed; 30 sec after injection purging was initiated.

The Varian model 3500 GC was equipped with a 30 m x 0.32 mm i.d. fused silica, wall-coated DB-5 capillary column (0.25 μm film thickness, J&W Scientific). Conditions of analysis were the same as employed with the HP instrument except that the linear velocity was 28 cm/sec at 300^{o}C.

Gas chromatography/nitrogen-phosphorus detection was performed using a Varian model 6000 GC and a 30 m x 0.32 mm i.d. fused silica,

wall-coated DB-1 capillary column (0.25 μm film thickness, J&W Scientific). The carrier gas was helium (linear velocity 31 cm/sec at 300°C) and the temperature increased from 70 to 300°C and 10°C/min beginning 1 min after injection. The oven temperature was maintained at 300°C for 20 min and the injector and detector temperatures were maintained at 270 and 300°C respectively. The injector was purged with helium 1 min after the injection of 0.5 μL of sample. The needle residence of the syringe in the injector was maintained at 30 sec via the autosampler.

Gas chromatography/mass spectrometry (GC/MS) was performed by interfacing a HP model 5890A GC to a HP model 5970 quadrupole mass spectrometer. Injection of 2 μL of sample was made into the GC (via a splitless injector) onto a 12.5 m x 0.2 mm i.d. fused silica, wall-coated HP-1 capillary column obtained from HP. The carrier gas was helium (linear velocity was 35.5 cm/sec at 300°C), and the temperature was increased from 40 to 300°C at 10°C/min beginning 1 min after injection. The oven temperature was maintained at 300°C for 8 min, and the injector, transfer line and ion source were maintained at 250, 300 and 220°C respectively. Data was acquired 2 min after injection using a HP model 59970C data system. Mass spectral scans (from 35 to 350 amu) were obtained every 1.36 seconds.

4.2.9. High Pressure Liquid Chromatography

Analysis for 6,7- and 6,8-DMQ in water was performed using a

Waters (Millipore Corp., Milford, MA) high pressure liquid chromatograph. The system consisted of two M6000 pumps, a model 680 solvent programmer, a model 710B autosampler, and a model 450 variable wavelength detector. Separations were performed on a 15 cm x 4.6 mm i.d. Supelcosil LC-PAH, 5-μm column (Supelco, Inc.). Analyses were performed isocratically using acetonitrile (70%) and ammonium phosphate buffer (pH 6.5). Flow rate was maintained at 3.0 mL/min and absorbance was monitored at 233 nm. Following injection of 15 to 20 μL of sample, chromatograms were recorded and integrated using a Hewlett-Packard model 3388A data system.

4.3. Results

4.3.1. Method Evaluation

Recovery of 6,7-DMQ from fortified tissue (liver, muscle and carcass) is summarized in table 4-1.

From these results it is apparent that acceptable levels of recovery for 6,7-DMQ from fortified fish tissue samples was obtained. The high recovery of total solutes from the liver sample may be attributed to the non-specificity of the GC/FID method used and to the co-elution of biogenic material. However the level of this interference (obtained when analyzing control samples) was not so high as to warrant further sample cleanup or reanalysis by a more specific method such as gas chromatography/nitrogen-phosphorus detection

(GC/NPD).

Analysis of tissue (muscle, liver, and carcass) as well as bile was performed primarily using GC/FID. Although this method of analysis is not as selective for quinoline derivatives as GC/NPD the extracts obtained were sufficiently clean in the region of the chromatogram where 6,7-DMQ and 6,8-DMQ appeared that this method was found to be more practical. Reference to figures 4-1 to 4-5 illustrate this logic. The figures contain chromatograms obtained from tissue samples (muscle, liver and carcass) fortified with 6,7-DMQ and 6,8-DMQ as well as bile obtained from fish exposed to these chemicals. These GC/FID chromatograms were compared to control tissue and bile samples and it was concluded that in most cases the samples were sufficiently clean that GC/FID analysis was practical. The only exception was fish liver in which a false positive was observed for 6,7-DMQ in the control sample. This was attributed to co-eluting biogenic material. Although the level of interference was observed to be small (1 μg/g) liver samples obtained from fish exposed to 6,7-DMQ and which had depurated were analyzed by GC/NPD.

Another reason why GC/FID analysis was preferred over GC/NPD, can be deduced from table 4-2 which is a comparison of the precision and accuracy obtained with these methods for the analysis of reference solutions containing 6,7-DMQ and 6,8-DMQ.

These results were obtained by analyzing replicate solutions containing 6,7-DMQ and 6,8-DMQ during analysis of tissue samples (liver, muscle, and carcass) by GC/NPD and GC/FID. The regime employed specified the analysis of one reference solution after every third

tissue sample (muscle and carcass) by GC/FID and the analysis of one reference solution after every liver sample using GC/NPD. From this table it is apparent that GC/FID analysis was the more accurate since the observed relative error was less than that observed with GC/NPD.

4.3.2. Tissue Distribution of 6,7-Dimethylquinoline in Fish

Table 4-3 is a summary of the tissue distribution observed for fish exposed to 6,7-dimethylquinoline with no depuration.

Mean exposure concentrations of 6,7-DMQ were determined by HPLC analysis of aquarium water obtained at regular time intervals during exposure (n=6-9).

The tissue distribution observed for fish exposed to 6,7-DMQ after depuration for 69 - 96 h is revealed in table 4-4.

As mentioned previously mean exposure concentrations were determined by analyzing water samples (taken during exposure, n=6-9) using HPLC.

From table 4-3 it is evident that 6,7-DMQ is readily taken up by fish and biocentrated from water in muscle, liver, bile and other tissue (carcass). Mean bioconcentration factors (from water, n=3) were determined to be 21, 18, 6 and 14 for bile, liver, muscle and carcass respectively. These were calculated by dividing the mean tissue concentrations by the mean exposure concentrations (table 4-3).

From table 4-4 it is also evident that 6,7-DMQ is readily depurated. No detectable levels of 6,7-DMQ were observed in samples of liver and bile taken from fish which had been exposed to this chemical

and depurated for 69 - 96 h. Detection limits were estimated to be 0.05 μg/g for carcass and muscle and 0.5 μg/g and 10 μg/g for liver and bile respectively using GC/FID analysis. The varying detection limits are a reflection of limited sample size (20 g for muscle and carcass and 2 g and 0.1 g for liver and bile respectively) and not methodology differences.

Despite depuration (69 - 96 h), 6,7-DMQ was still found to be present in muscle and carcass at detectable levels. Mean values were determined to be 0.54 and 0.48 μg/g for muscle and carcass respectively (n=4).

4.3.3. Characterization of 6,7-Dimethylquinoline Metabolites in Exposed Trout

Extraction of bile (with chloroform-methanol), obtained from fish exposed to 6,7-DMQ, and analysis of the extract using GC/FID revealed the presence of mainly parent compound (figure 4-6; upper chromatogram). However, enzymatic hydrolysis of the aqueous phase derived from the previous extraction, followed by extraction and GC/FID analysis of the hydrolyzate revealed the presence of two large GC peaks (figure 4-6; bottom chromatogram) which suggests the presence of conjugated metabolites.

Analysis of bile obtained from fish exposed to 6,7-DMQ, after enzymatic hydrolysis and extraction, by GC/MS produced three important mass spectra (figure 4-7). The spectrum appearing at 12.348 min was

correlated with the parent compound as the observed spectrum and retention time matched that observed for reference material. Mass spectra obtained at retention times of 15.588 and 15.646 min (figure 4-7) were identical. Further mass spectra were retrieved and studied from various points along the broad unresolved peak observed in the total ion chromatogram (figure 4-7; top). No differences in the observed spectra were apparent. Analysis by GC/FID did reveal two distinct peaks so it is apparent that the major metabolites of 6,7-DMQ consist of at least two compounds with similar mass spectra.

In order to ascertain whether or not these metabolites were phenolic and to obtain further structural information, an extract containing the above metabolites was subjected to an aqueous acetylation. GC/FID analysis of the resulting extract revealed that no reaction had taken place as would be expected with alcohols. Phenolic hydroxyl groups would acetylate under the conditions employed (Coutts *et al.*, 1979). For confirmation, these results were compared to those obtained from an attempted acetylation of benzyl alcohol. GC/MS analysis of the resulting solution again showed that no acetylation of benzyl alcohol occurred.

In order to provide further structural information for the metabolites obtained from 6,7-DMQ an extract containing these metabolites was also subjected to acylation using pentafluorbenzoyl chloride. Figure 4-8 is a summary of the results obtained when the acylated extract containing the metabolites was analyzed by GC/MS. Two well resolved GC peaks were observed (upper total ion chromatogram) and the corresponding mass spectra were retrieved and found to be

similar.

The above results were compared to those obtained from the pentafluorobenzoylation of benzyl alcohol. GC/MS analysis of the ensuing products revealed that benzyl alcohol partially reacted (or reacted then partially hydrolyzed). GC/MS analysis of metabolites obtained from the bile of fish exposed to 6,7-DMQ before and after pentafluorbenzoylation revealed the presence of two major metabolites with similar mass spectra. Concentrations of these metabolites were determined in bile of fish exposed to 6,7-DMQ using GC/FID and 1-hydroxymethylnaphthalene as a quantitation standard. Since the two major metabolites were not well resolved on the GC column, the total area observed for the two compounds was employed in the calculation and therefore the combined concentration was reported. Table 4-5 summarizes these results.

From these results it can be seen that depurating fish had higher levels of metabolites in their bile after exposure to 6,7-DMQ than did fish who were exposed to this chemical but were not allowed to depurate the chemical. It is also interesting to note that the concentration of metabolites varied as a function of feeding during depuration presumably due to bile clearance. Although the lowest concentration of metabolites was observed for a fish which was allowed to feed during depuration (Table 4-5; 811 μg/g), the highest concentration was also observed for a fish which was allowed to feed during depuration (1494 μg/g). This might be explained by the fact that the fish were very stressed during exposure and this stress may have had an effect on bile clearance.

Analysis of liver for conjugated metabolites using GC/FID revealed no detectable levels which suggests that these metabolites are quickly excreted into the bile.

4.3.4. Distribution of 6,8-Dimethylquinoline in Fish Tissue

The tissue distribution of 6,8-DMQ in fish which were exposed to this chemical in water for 8 h and then sacrificed (i.e. no depuration) is shown in table 4-6.

Tissue distribution for 6,8-DMQ following exposure and depuration is shown in table 4-7.

From table 4-6 it is apparent the 6,8-DMQ like 6,7-DMQ is readily taken up by the tissues studied during exposure. Mean bioconcentration factors from water were determined to be 23, 20, 13, and 25 for bile, liver, muscle and carcass respectively. Again these were determined by dividing the mean exposure concentration into the mean tissue concentrations (table 4-6).

Mean exposure concentrations of 6,8-DMQ in water were determined by HPLC analysis of aquarium water obtained at regular time intervals during exposure (n=6-9).

Although 6,8-DMQ is readily bioconcentrated in tissue after exposure, from table 4-7 it is evident that this chemical is also eliminated from tissue during depuration. The mean concentration of 6,8-DMQ observed in muscle after depuration was found to be 0.49 μg/g. This mean concentration was similar to that observed for fish muscle

obtained from fish exposed to 6,7-DMQ after depuration (0.54 μg/g; table 4-4). One noteworthy observation is the mean concentration of 6,8-DMQ in carcass of fish after exposure and depuration (table 4-7). This mean concentration (3.19 μg/g) is significantly higher ($p<0.05$) than that observed for carcass obtained from fish after exposure to 6,7-DMQ and depuration (0.48 μg/g - table 4-4).

4.3.5. Identification of Metabolites in Fish Exposed to 6,8-Dimethylquinoline

GC/MS analysis of bile obtained from fish exposed to 6,8-DMQ after enzymatic hydrolysis and extraction revealed the presence of four GC peaks (top of figure 4-9). The mass spectrum for the peak at retention time 11.432 min (figure 4-9; bottom) was retrieved and concluded to be parent compound because the observed mass spectrum and GC retention time were similar to that obtained for 6,8-DMQ. Mass spectra for the remaining peaks or metabolites were retrieved and are shown in figure 4-10. These peaks were not found to be present in control fish and appeared in exposed fish bile only after enzymatic hydrolysis indicating that they are conjugated metabolites. The metabolites appearing at the retention times of 14.674 and 14.853 min possessed similar mass spectra; however the mass spectrum obtained from the metabolite appearing at retention time 14.993 min was different from the other metabolites. The three metabolite spectra all contained a strong molecular ion with a m/z of 173 amu.

In order to obtain further structural information a bile extract

containing these metabolites was subjected to acetylation. Figure 4-11 illustrates the total ion chromatogram obtained for this acetylated extract during GC/MS analysis. Two prominent peaks in the retention window of 14 to 16 min were observed. Mass spectra for these two peaks were retrieved and are shown in figure 4-12. The mass spectrum of the peak at 15.077 min was similar to that obtained for one of the metabolites illustrated in figure 4-10 which had virtually the same retention time (14.993 min) (figure 4-10, bottom). This observation indicates that this metabolite did not acetylate and therefore was not phenolic.

An extract obtained from bile of a fish exposed to 6,8-DMQ, which contained metabolites, was also subjected to acylation with PFBC in order to obtain further structural information. The GC/MS analysis of this extract is summarized in figure 4-13. As in the case with acetylation, two major GC peaks were observed (top of figure 4-13). Mass spectra were retrieved for these peaks occurring at retention times of 19.785 and 20.435 min. respectively and are displayed in figure 4-13 (bottom portion). Study of these mass spectra reveals that the compounds represented by the GC peaks are different.

In order to follow the concentrations of metabolites in bile as a function of depuration, metabolites observed in fish exposed to 6,8-DMQ were quantitated in bile using GC/FID and 8-hydroxyquinoline as a quantitation standard. Results are summarized in table 4-8. These results should also be read in conjunction with table 4-7 which shows that fish identified by weights of 215 and 166 g were fed during depuration and that the fish weighing 206 g was not fed during

depuration.

4.4 Discussion

4.4.1 Uptake and Elimination of 6,7-Dimethylquinoline

Exposure experiments conducted with 6,7-DMQ revealed that this chemical is readily taken up by rainbow trout and concentrated in the bile, liver, muscle and remaining tissue (carcass). Mean concentration factors (n=3) from water were determined to be 21, 18, 6, and 14 for bile, liver, muscle and carcass respectively. These results are consistent with those reported by Bean et al (1985) for the uptake and elimination of quinoline. These workers found the relative concentrations of quinoline after 24 h exposure to be gall bladder > eye > gut > kidney > liver > gill > muscle. While 6,7-DMQ was readily absorbed by fish tissues during exposure, it is also readily eliminated during periods of depuration. This compound was not detected in bile and liver after 69 h of depuration. Furthermore mean concentration levels in muscle and carcass were reduced to 0.54 and 0.48 μg/g respectively. These results are also consistent with those obtained by Bean et al (1985) who observed a significant decrease in quinoline concentrations in b e, liver, kidney, muscle, gut, gill, and eye after 24 h depuration.

4.4.2. Characterization of 6,7-Dimethylquinoline Metabolites in Exposed Trout

When bile obtained from fish exposed to 6,7-DMQ was extracted before and after enzymatic hydrolysis, the presence of metabolites was revealed only after enzymatic hydrolysis (figure 4-5). Two distinct GC peaks were observed with similar mass spectra. The molecular ions in spectra of both metabolites were of m/z 173, whereas the molecular ion of 6,7-DMQ was of m/z 157. Thus, both metabolites were mono-oxygenated derivatives of the substrate, 6,7-DMQ. This liberation from bile only after hydrolysis suggests that the metabolites were conjugates with glucuronide or sulfate. Attempted acetylation of the enzymatically hydrolyzed metabolites followed by GC analysis revealed that no reaction had taken place. This observation indicated that the primary metabolites were conjugated alcohols and not phenols since it was known that phenols are acetylated under the conditions employed (Coutts *et al.*, 1979), and it was confirmed that the reference compound benzyl alcohol did not undergo acetylation under these conditions.

Inspection of the mass spectra (figure 4-7) indicated a ready loss of water from the molecular ions to generate the base peak of m/z 155. This loss of water can be explained by an ortho effect involving adjacent methyl and hydroxymethyl groups (Beynon *et al*,1968). The presence of an alcohol moiety at the 6 or 7 position of the quinoline nucleus can explain such a mass spectrum. Our conclusion

is that enzymatic hydrolysis of the bile released the two primary metabolites, now identified as 7-hydroxymethyl-6-methylquinoline and 6-hydroxymethyl-7-methylquinoline. These two isomers would be expected to generate similar mass spectra. A possible fragmentation mechanism, common to both mass spectra, is illustrated in figure 4-14. This mechanism was substantiated by comparisons with known fragmentations of benzyl alcohol (Beynon *et al*, 1968) and alkylquinolines (Draper and MacLean, 1968).

Further evidence suggesting that the major primary metabolites derived from the metabolism of 6,7-DMQ were alcohols was provided by reaction with PFBC. This acylation resulted in the formation of two distinct GC peaks with longer retention times (figure 4-8). Furthermore, the mass spectra obtained for these two GC peaks were observed to be similar. While reaction of the primary metabolites with PFBC was apparent, it was also observed that this reaction was not quantitative because the presence of unreacted metabolites was also observed by GC/MS analysis. This observation paralleled that of benzyl alcohol which did not derivatize quantitatively when reacted with PFBC under identical conditions. A likely fragmentation mechanism for the PFBC derivatives of the primary metabolites is shown in figure 4-15. The base peak of m/z 155 in the mass spectrum of the pentafluorobenzoylated metabolite is again the product of an ortho-effect, and indicative of an acylated alcohol and not of a phenol.

These results permit the postulation that the major metabolites of 6,7-DMQ are conjugated 6-hydroxymethyl-7-methylquinoline and 7-hydroxymethyl-6-methylquinoline. Because of the ease with which they

are hydrolyzed, the conjugates are clearly either sulfates or glucuronides and not mercapturic acid conjugates, such as reported by Bean *et al*, (1985) for quinoline.

Quantitation of metabolites was performed by analysis using a DB-1301 capillary column and 1-hydroxymethylnaphthalene as a quantitation standard. The metabolites separated much better on a DB-1301 capillary column than on a DB-5 capillary column on which 1-hydroxymethylnaphthalene tailed badly. 1-Hydroxymethylnaphthalene was used as a quantitation standard in the belief that it would have a detector response similar to those of 6-hydroxymethyl-7-methylquinoline and 7-hydroxymethyl-6-methylquinoline. These standards were not available to us. This assumption was made more credible when a GC/FID analysis on a DB-1301 capillary column showed that quinoline and naphthalene gave a similar GC/FID response (0.0720 and 0.0566 ng/unit area respectively).

In table 4-5 it is observed that metabolites are concentrated in the bile 347- to 710-fold above the exposure level after 7.5 h of exposure. After 7.5 h of exposure followed by 96 h of depuration the metabolites are concentrated in bile 854 fold above exposure levels, and after 9.5 h of exposure followed by 69 h of depuration metabolite concentrations were observed to be 1265- to 1494-fold higher than exposure levels. The concentration levels observed for the metabolites of 6,7-DMQ in bile are substantially higher than those reported for metabolites of quinoline in bile after exposure to radiolabelled quinoline. Bean *et al*, (1985) found bile levels of radioactivity were concentrated 370 to 420 times above exposure water concentrations. Our

finding, however, is in keeping with that of Melancon and Lech (1979) who observed greater bile-^{14}C to water-^{14}C ratios for fish exposed to ^{14}C-2-methylnaphthalene than for fish exposed to ^{14}C-naphthalene. This was attributed to the greater uptake of ^{14}C-2-methylnaphthalene. The mean bioconcentration factor (from water) for quinoline in whole body extracts obtained from exposed juvenile rainbow trout was 3.73 ± 0.43 (Bean *et al*, 1985). In the current study, the mean bioconcentration factor for 6,7-DMQ in fish muscle obtained from exposed rainbow trout was 5.86 ± 1.68 (n=3). It is concluded that 6,7-DMQ metabolites are more highly concentrated in bile of exposed fish than metabolites of quinoline itself.

4.4.3 Uptake and Elimination of 6,8-Dimethylquinoline

An inspection of table 4-6 reveals that 6,8-DMQ like 6,7-DMQ is readily taken up by fish tissues during exposure to these quinoline derivatives. Mean bioconcentration factors from water were determined to be 23, 20, 13 and 25 for bile, liver, muscle and carcass respectively.

Although 6,8-DMQ is readily bioconcentrated in tissue after exposure, it is evident, from table 4-7, that this chemical is also eliminated from tissue during depuration. The mean concentration of 6,8-DMQ observed in muscle after depuration was found to be 0.49 μg/g, a value similar to that observed for fish muscle obtained from fish exposed to 6,7-DMQ after depuration (0.54 μg/g - table 4). One noteworthy observation is the mean concentration of 6,8-DMQ in carcass

of fish after exposure and depuration (table 4-7). This mean concentration (3.19 μg/g) is significantly higher than that observed for carcass obtained from fish after exposure to 6,7-DMQ and depuration (0.48 μg/g; table 4-4). This result indicates that these isomeric DMQs have different tissue distribution properties.

4.4.4. Identification of Metabolites in Fish Exposed to 6,8-Dimethylquinoline

GC/MS analysis of bile obtained from fish exposed to 6,8-DMQ after enzymatic hydrolysis and extraction revealed the presence of 3 poorly resolved GC peaks. These GC peaks appeared only after enzymatic hydrolysis of the bile indicating that they were derived from glucuronide or sulfate conjugates. When mass spectra were retrieved for these GC peaks, the spectra of those with retention times of 14.674 and 14.853 min were almost identical, whereas the peak with a retention time of 14.993 min had a unique mass spectrum (figure 4-10). The hydrolyzed extract containing metabolites, was acetylated and reanalyzed by GC/MS. Two GC peaks were obtained, and their mass spectra were recorded. This revealed that the peak with retention time 15.613 was acetylated while the peak with retention time 15.077 min was not (figure 4-12). The mass spectrum of the peak of retention time 15.077 min (figure 4-12) was identical to the one observed before acetylation at retention time 14.993 min (figure 4-10). These observations suggest that the primary metabolites consist of alcohols and phenols. Since the GC peak appearing at 14.993 min (figure 4-10)

did not acetylate it is concluded that this metabolite is 8-hydroxymethyl-6-methylquinoline or its isomer, 6-hydroxymethyl-8-methylquinoline. The GC peaks appearing at 14.674 min and 14.853 min (figure 4-10) are structurally related phenols which are acetylated to products that produce a single unresolved GC peak. The GC peak appearing at RT 14.993 (figure 4-9; top) and RT 15.077 (figure 4-11) is likely that of 6-hydroxymethyl-8-methylquinoline. This structure was deduced from the results obtained by analyzing bile of fish exposed to two reference compounds, 6-methylquinoline and 8-methylquinoline. Mass spectra were obtained and it was revealed that the major metabolite present in the bile of fish exposed to 6-methylquinoline had a mass spectrum with features similar to that observed for 6,8-dimethylquinoline (figure 4-16). The common feature was the presence of a strong $(M-29)^+$ ion, believed to arise by the expulsion of a CHO radical. Furthermore, comparison of the mass spectrum obtained from the bile of fish exposed to 8-methylquinoline to that of a reference spectrum of 8-quinolinemethanol (Eight Peak Index, 1986) revealed that the mono-oxygenated metabolite of 8-methylquinoline was not the hydroxymethyl compound. With this evidence it was deduced that the metabolite appearing at retention time 15.077 min (figure 4-11) was 6-hydroxymethyl-8-methylquinoline. A postulated fragmentation mechanism is shown in figure 4-17. The mass spectrum (figure 4-12) observed for the acetylated GC peak appearing at RT 15.613 min (figure 4-11) is rationalized in figure 4-18. This peak is likely phenolic, good candidates are 3-hydroxy-, 5-hydroxy- or 7-hydroxy-6,8-dimethylquinoline.

In order to obtain further structural information an extract obtained from bile of a fish exposed to 6,8-DMQ, which contained primary metabolites, was also subjected to acylation with PFBC. As in the case with acetylation, two major GC peaks were observed (figure 4-13; top). Mass spectra were retrieved for the peaks occurring at retention times of 19.785 and 20.435 min and are displayed in figure 4-13 (bottom portion). The observed spectra were unique. The mass spectrum of the peak of RT 19.785 min is due to co-eluting phenols, likely 5-hydroxy-6,8-dimethylquinoline and 7-hydroxy-6,8-dimethylquinoline. Two phenols are suggested because the extract from enzymatically hydrolyzed bile obtained from fish exposed to 6,8-DMQ produced three GC peaks (figure 4-9; top), which, on acetylation, were reduced to two GC peaks (figure 4-11), one of which was concluded to be 6-hydroxymethyl-8-methylquinoline. This observation suggests that the two phenols present formed acetates which chromatographed as an unresolved GC peak. Studies conducted by Bean *et al* (1985) revealed that 2- and 4-hydroxyquinolines do not form acetate derivatives. Therefore, the two phenols of interest must be two of the three possibilities, 5-hydroxy-, 7-hydroxy- or 3-hydroxy-6,8-dimethylquinoline. A proposed fragmentation mechanism to accommodate the observed mass spectrum (PFBC derivative - RT 19.785, figure 4-13) is shown in figure 4-19. The mass spectrum of the analyte of RT 20.435 is consistent with it being the PFBC derivative of 6-hydroxymethyl-8-methylquinoline. A proposed fragmentation mechanism is shown in figure 4-20.

In conclusion it is apparent that both 6,7-DMQ and 6,8-DMQ are

readily absorbed by fish tissue and metabolized. The major metabolites of 6,7-DMQ were shown to be alcohols whereas the major metabolites of 6,8-DMQ were shown to consist of an alcohol and at least two phenols. After depuration for up to 96 h along with feeding, high levels (>1000 μg/g) of metabolites were still present in the bile of fish. Since bioabsorbed metabolites are frequently the source of toxicity (Bean *et al*, 1985 and Krahn *et al*, 1984) such a finding may be cause for concern and should be investigated further.

4.5. TABLES

Table 4-1 Recovery of 6,7-Dimethylquinoline From Fortified Fish Tissue

Tissue type	Mean Recovery (%)	Replicate Analyses	Range of Recoveries (%)	Fortification Level (μg/g)
muscle	98	4	90 - 106	1.15
carcass	97	2	95 - 100	1.15
liver	115	1		6.70

Table 4-2 Precison and Accuracy Comparison for GC/FID and GC/NPD

	6,7-DMQ	6,8-DMQ
Amount added, A (μg/mL)	22.98	27.72
Amount found by GC/FID, x (mean)	22.89	27.71
Number of observations, n	19	19
Standard deviation, s	1.70	1.81
Relative standard deviation, s/x . 100	7.39	6.53
Relative error, A-x/A .100	0.04	0.39
Amount found by GC/NPD	22.09	28.27
Number of observations	7	7
Standard deviation, s	1.26	1.55
Relative standard deviation	5.70	5.48
Relative error	3.87	1.98

Table 4-3 Tissue Distribution of 6,7-Dimethylquinoline in Fish (No Depuration)

Replicate number	1	2	3
Weight of fish (g)	164	180	183
Length of fish (mm)	242	247	247
Exposure time (h)	7.5	7.5	7.5
Mean exposure conc. (mg/L)	1.00	1.10	0.98
Exposure conc. range (mg/L)	0.94-1.07	1.02-1.20	0.92-1.04
Bile concentration (μg/g)	17.72	24.2	24.1
Liver concentration (μg/g)	19.4	17.2	20.2
Muscle concentration (μg/g)	4.75	8.58	4.94
Carcass concentration (μg/g)	20.1	17.0	5.6

Table 4-4 Tissue Distribution of 6,7-Dimethylquinoline in Fish After Depuration

Replicate number	1	2	3	4
Weight of fish (g)	308	269	188	308
Length of fish (mm)	288	277	210	284
Exposure time (h)	9.5	9.5	9.5	7.5
Depuration time (h)	69	69	69	96
Mean exposure conc. (mg/L)	0.94	0.98	1.00	0.95
Exp. conc. range (mg/L)	0.89-1.05	0.90-1.12	0.96-1.06	0.88-1.07
Bile concentration (μg/g)	ND	ND	ND	ND
Liver concentration (μg/g)	ND	ND	ND	ND
Muscle concentration (μg/g)	0.58	0.41	0.68	0.50
Carcass concentration (μg/g)	0.44	0.37	0.55	0.57
Fed during depuration	Y	N	Y	Y

Table 4-5 Concentration of Conjugated Metabolites Found in the Bile of Fish Exposed to 6,7-Dimethylquinoline

Exposure time (h)	Weight of fish (g)	Depuration time (h)	Mean Exposure Conc. (mg/L)	Concentration of metabolites (μg/g)
7.5	164	0	1.00	347
7.5	180	0	1.10	781
7.5	183	0	0.98	372
7.5	308	96	0.95	811
9.5	269	69	0.98	1240
9.5	188	69	1.00	1494

Table 4-6 Tissue Distribution of 6,8-Dimethylquinoline in Fish

(No Depuration)

Replicate number	1	2	3
Weight of fish (g)	235	265	208
Length of fish (mm)	268	267	259
Exposure time (h)	8	8	8
Mean exposure conc. (mg/L)	1.22	0.90	1.08
Exposure conc. range (mg/L)	1.08-1.39	0.78-1.06	0.96-1.26
Bile concentration (μg/g)	29.7	19.0	26.7
Liver concentration (μg/g)	26.2	16.8	21.0
Muscle concentration (μg/g)	12.9	16.0	12.4
Carcass concentration (μg/g)	11.6	31.6	37.2

Table 4-7 Distribution of 6,8-Dimethylquinoline in Fish Tissue After Exposure and Depuration

Replicate number	1	2	3
Weight of fish (g)	215	166	206
Length of fish (mm)	260	244	255
Exposure time (h)	7	7	7
Depuration time (h)	63	63	63
Mean exposure conc. (mg/L)	1.04	1.33	0.97
Exposure conc. range (mg/L)	0.94-1.26	1.25-1.50	0.87-1.12
Bile concentration (μg/g)	1.11	3.8	7.0
Liver concentration (μg/g)	0.90	0.49	0.61
Muscle concentration (μg/g)	0.54	0.45	0.47
Carcass concentration (μg/g)	1.86	1.99	5.71
Fed during depuration	y	y	n

Table 4-8 Concentration of Conjugated Metabolites Found in the Bile of Fish Exposed to 6,8-Dimethylquinoline

Exposure time (h)	Weight of fish (g)	Depuration time (h)	Mean Exposure Conc. (mg/L)	Concentration of metabolites (μg/g)		
				#1	#2	#3
8	235	0	1.22	280	241	766
8	265	0	0.90	172	244	648
8	259	0	1.08	241	273	970
7	215	63	1.04	0	36	89
7	166	63	1.33	69	219	675
7	206	63	0.97	112	843	1049

4.6. FIGURES

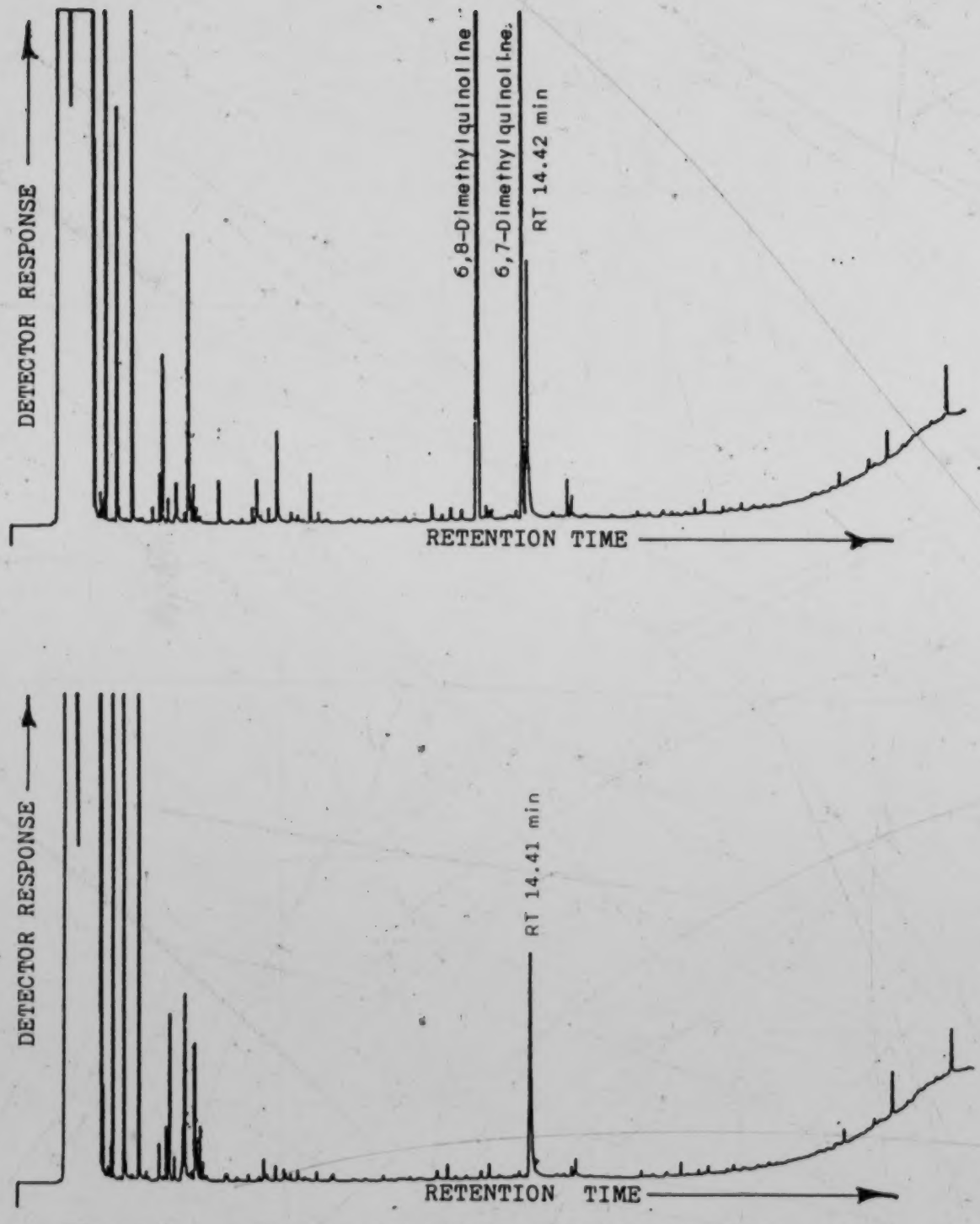

Figure 4-1 UPPER: FISH MUSCLE FORTIFIED WITH 6,7- AND 6,8-DIMETHYLQUINOLINE; LOWER: CONTROL FISH MUSCLE

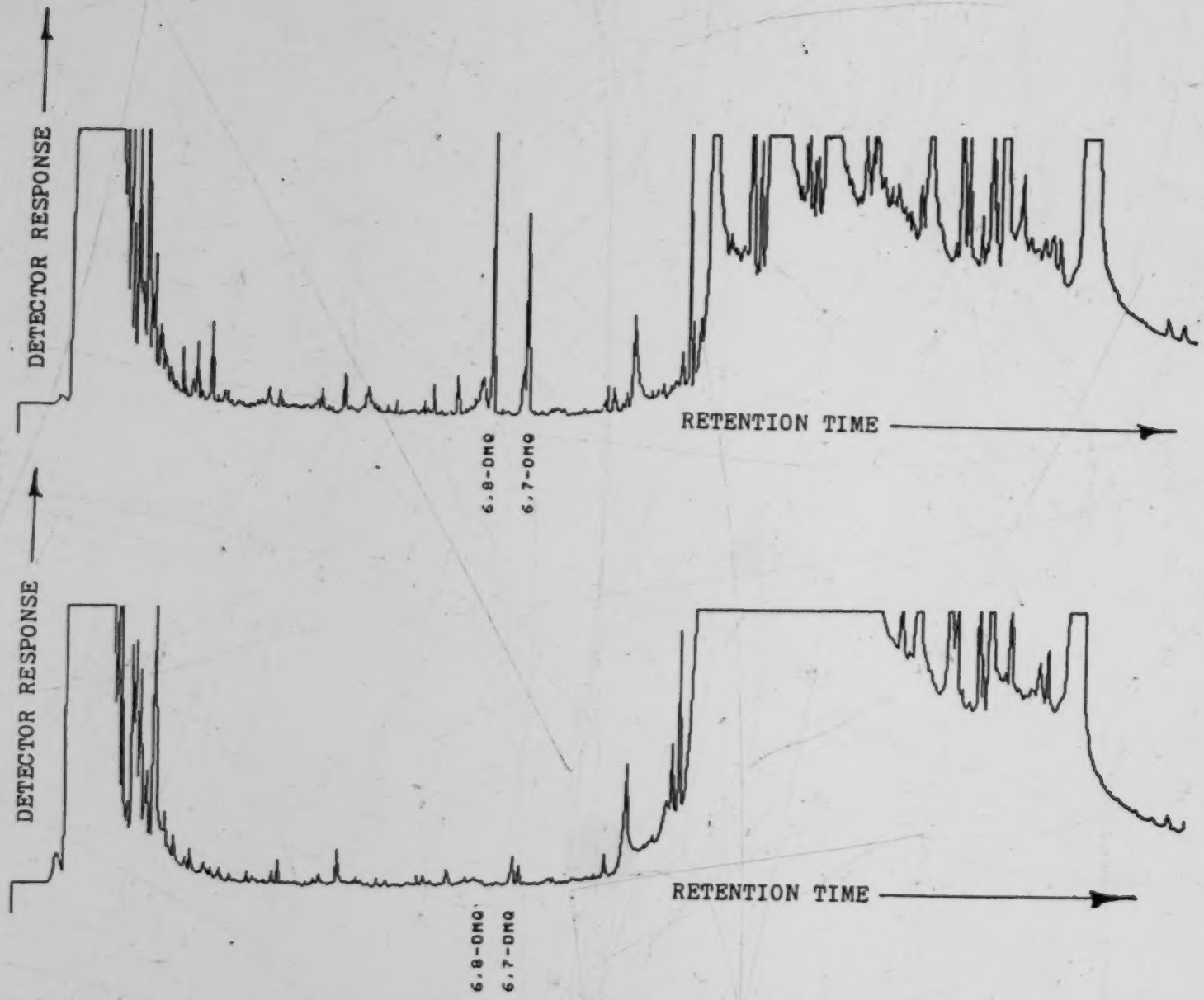

Figure 4-2 UPPER: FISH LIVER FORTIFIED WITH 6,7- AND 6,8-DIMETHYLQUINOLINE; LOWER: CONTROL FISH LIVER

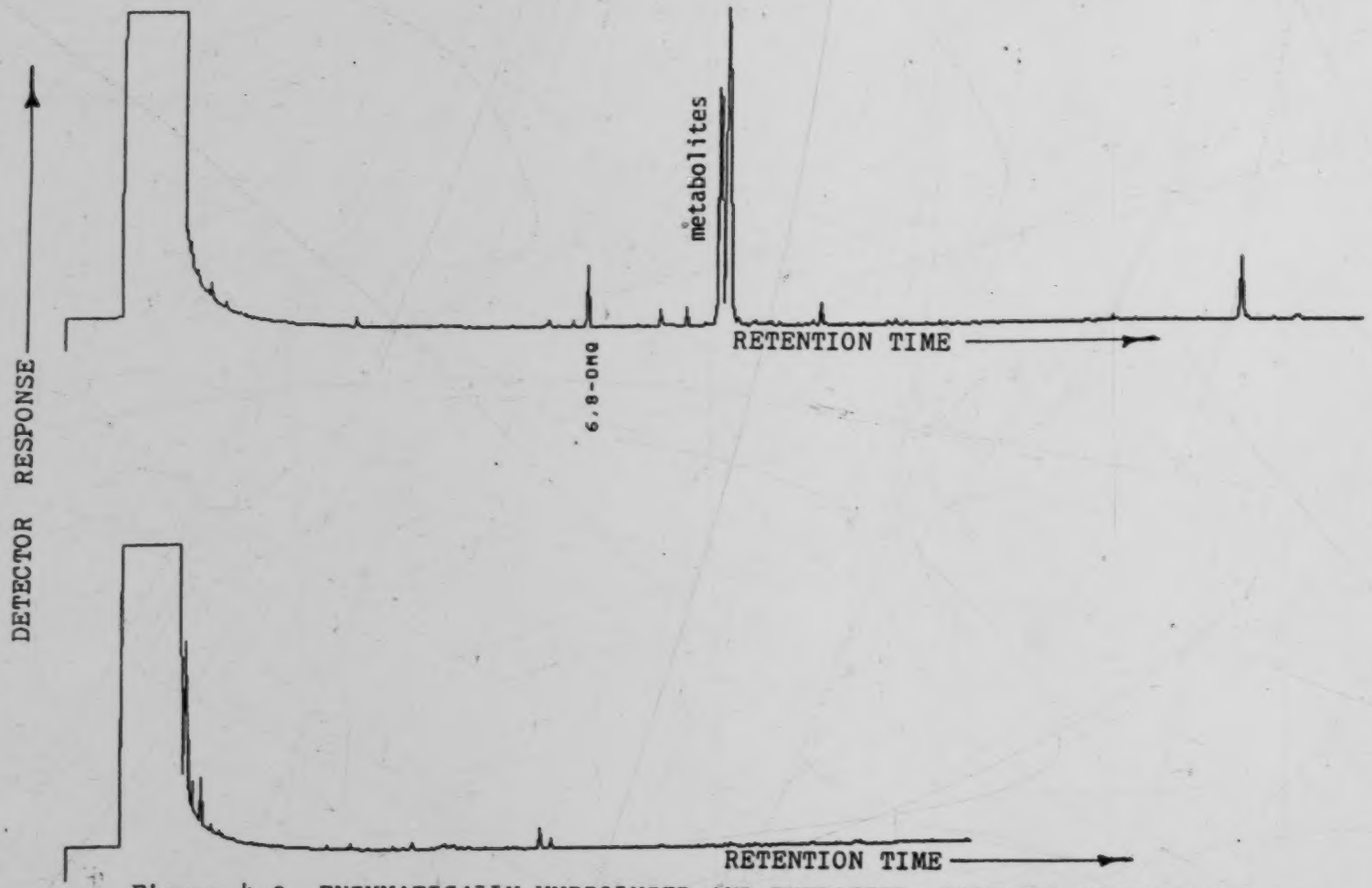

Figure 4-3 ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6,8-DIMETHYLQUINOLINE (UPPER FIGURE); ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM A CONTROL FISH (LOWER FIGURE)

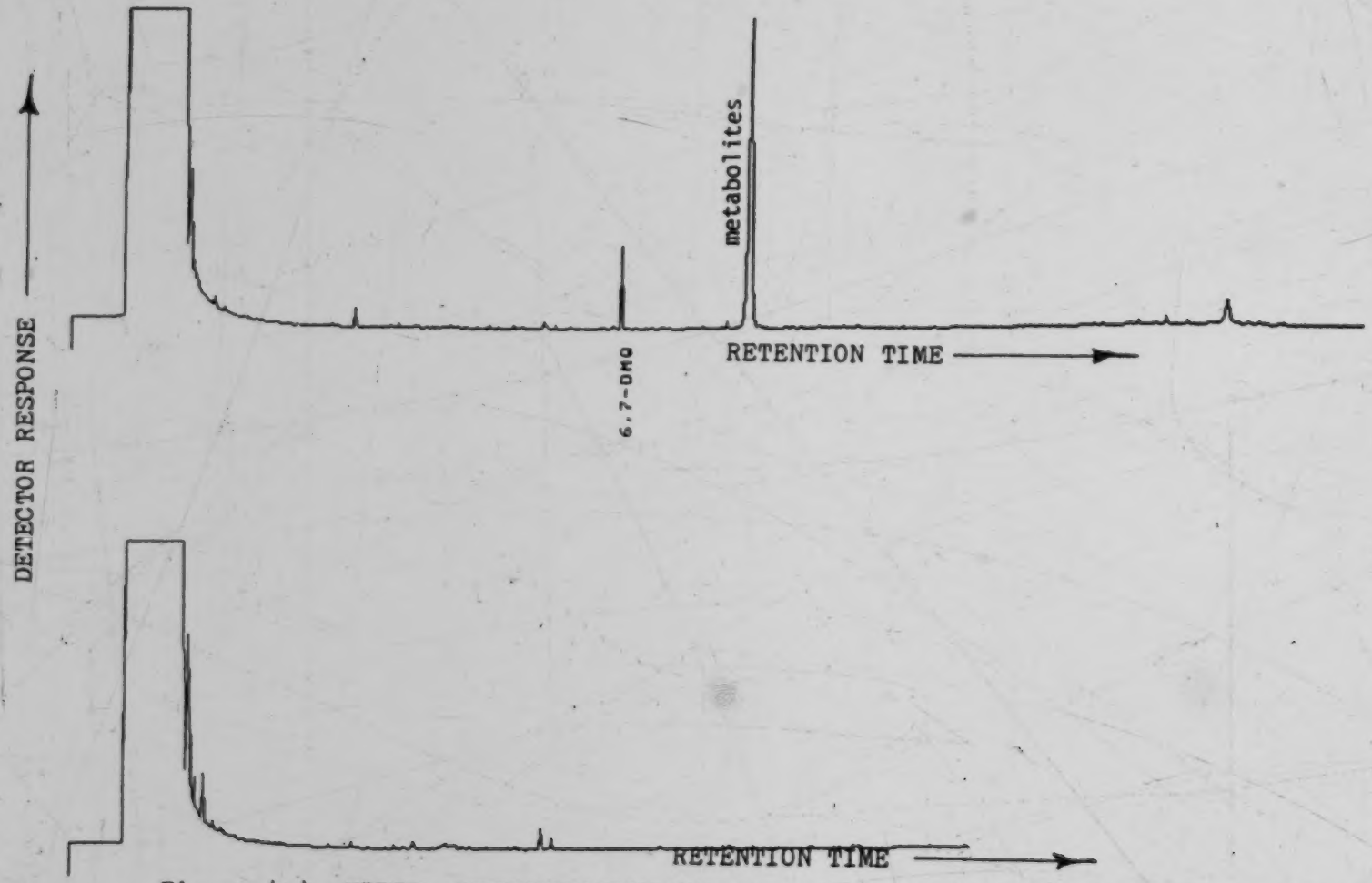

Figure 4-4 UPPER: ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6,7-DIMETHYLQUINOLINE; LOWER: ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM A CONTROL FISH

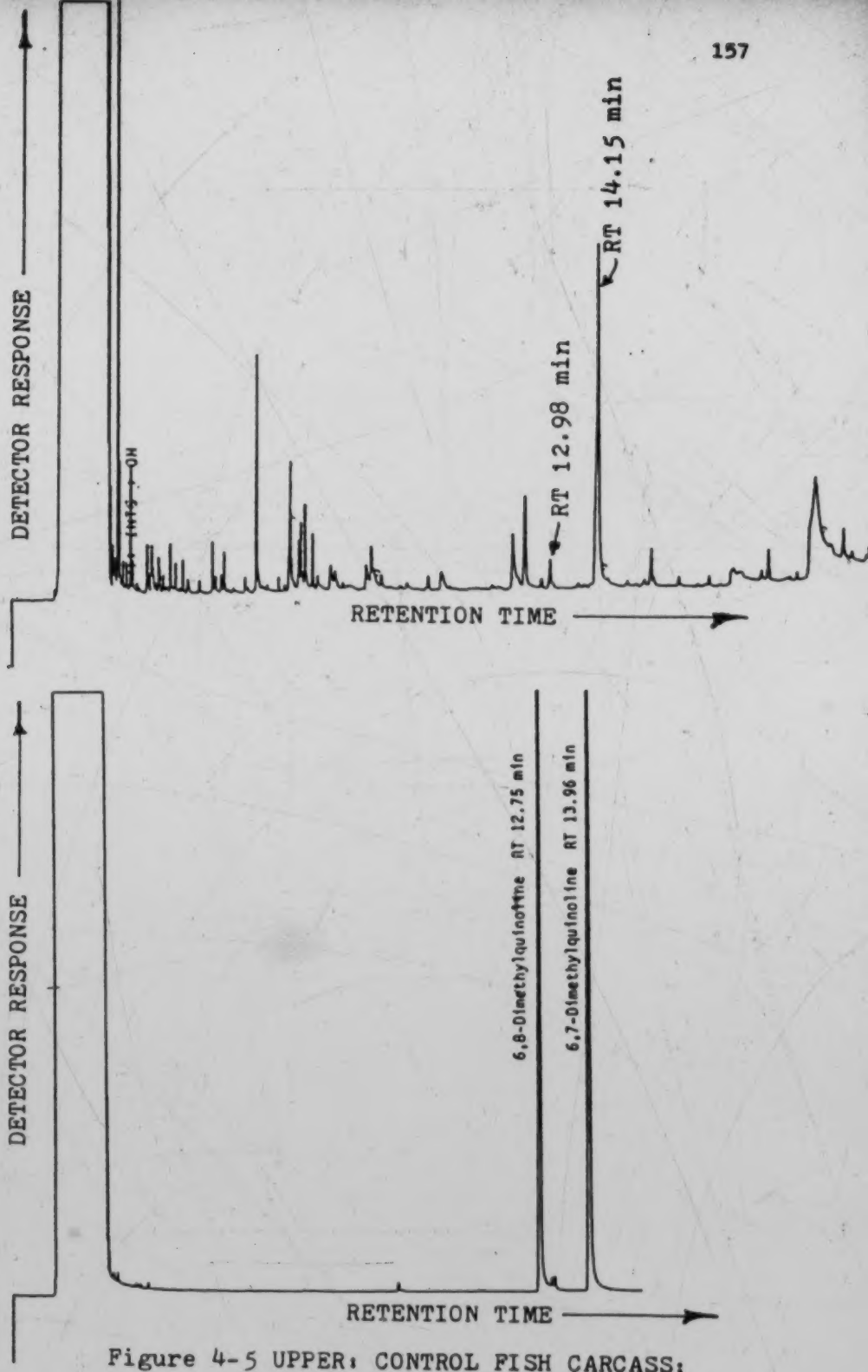

Figure 4-5 UPPER: CONTROL FISH CARCASS; LOWER: SOLUTION CONTAINING 6,7- AND 6,8-DIMETHYLQUINOLINE

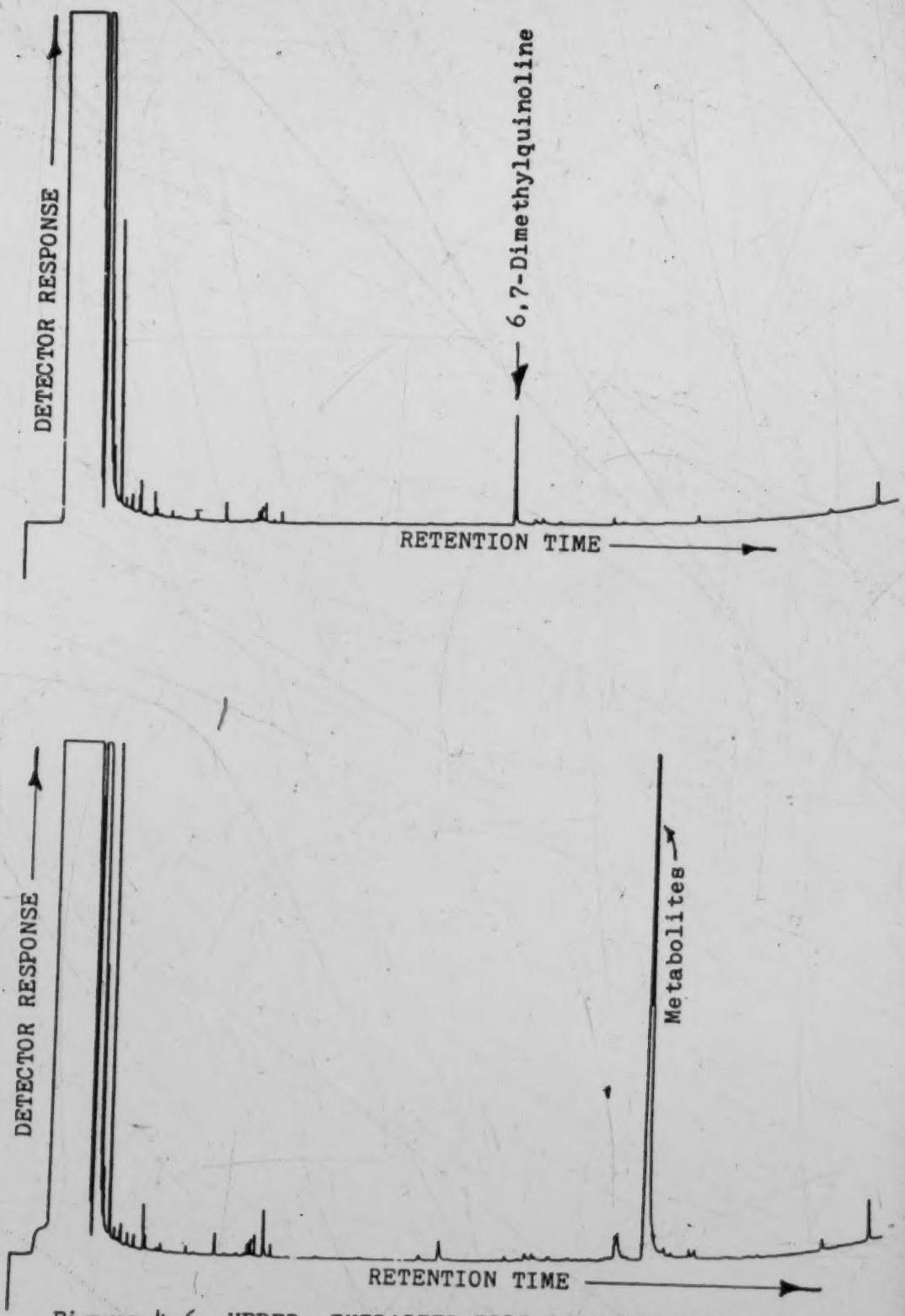

Figure 4-6 UPPER: EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6,7-DIMETHYLQUINOLINE; LOWER: AQUEOUS PHASE FROM THE SAME EXTRACTION AFTER ENZYMATIC HYDROLYSIS AND FURTHER EXTRACTION

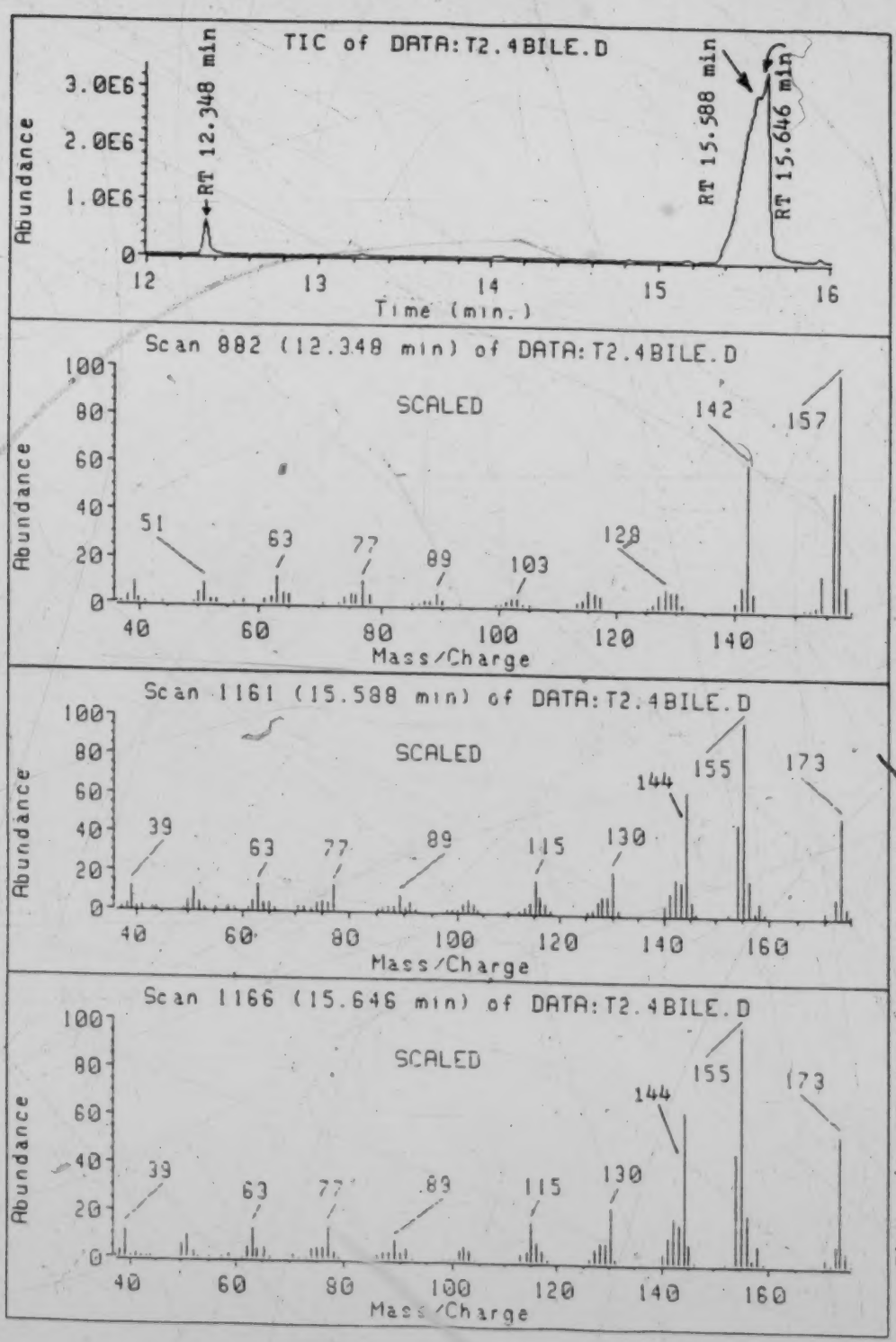

Figure 4-7 UPPER: TOTAL ION CHROMATOGRAM OBTAINED FROM ENZYMATICALLY HYDROLYSED AND EXTRACTED BILE OF FISH EXPOSED TO 6,7-DIMETHYLQUINOLINE; LOWER: MASS SPECTRA FOR PEAKS APPEARING AT RETENTION TIMES 12.348, 15.588 AND 15.646 MIN.

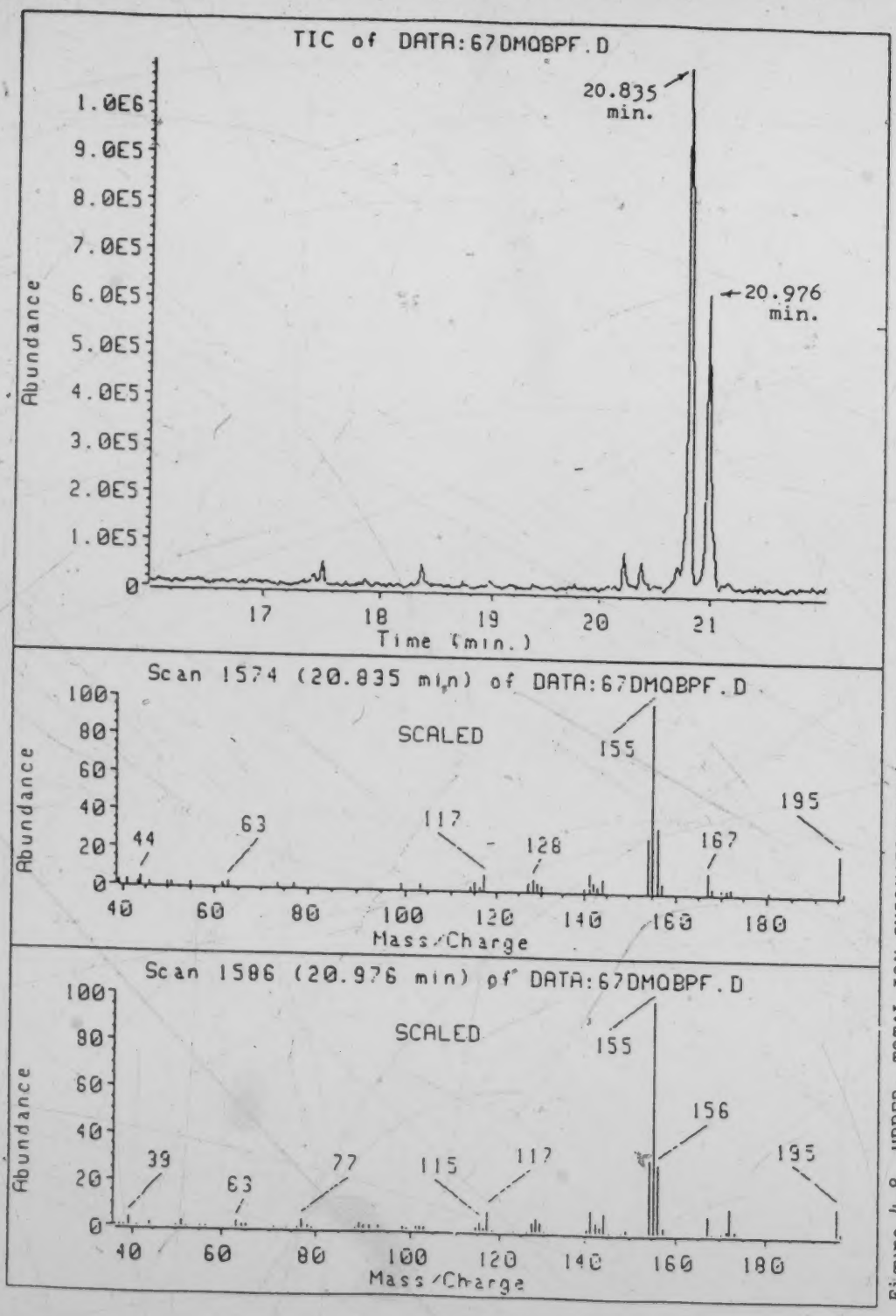

Figure 4-8 UPPER: TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED, EXTRACTED AND ACYLATED (WITH PFBC) BILE OBTAINED FROM FISH EXPOSED TO 6,7-DIMETHYLQUINOLINE; LOWER: MASS SPECTRA FOR GC PEAKS APPEARING AT RETENTION TIMES 20.835 AND 20.976 MIN.

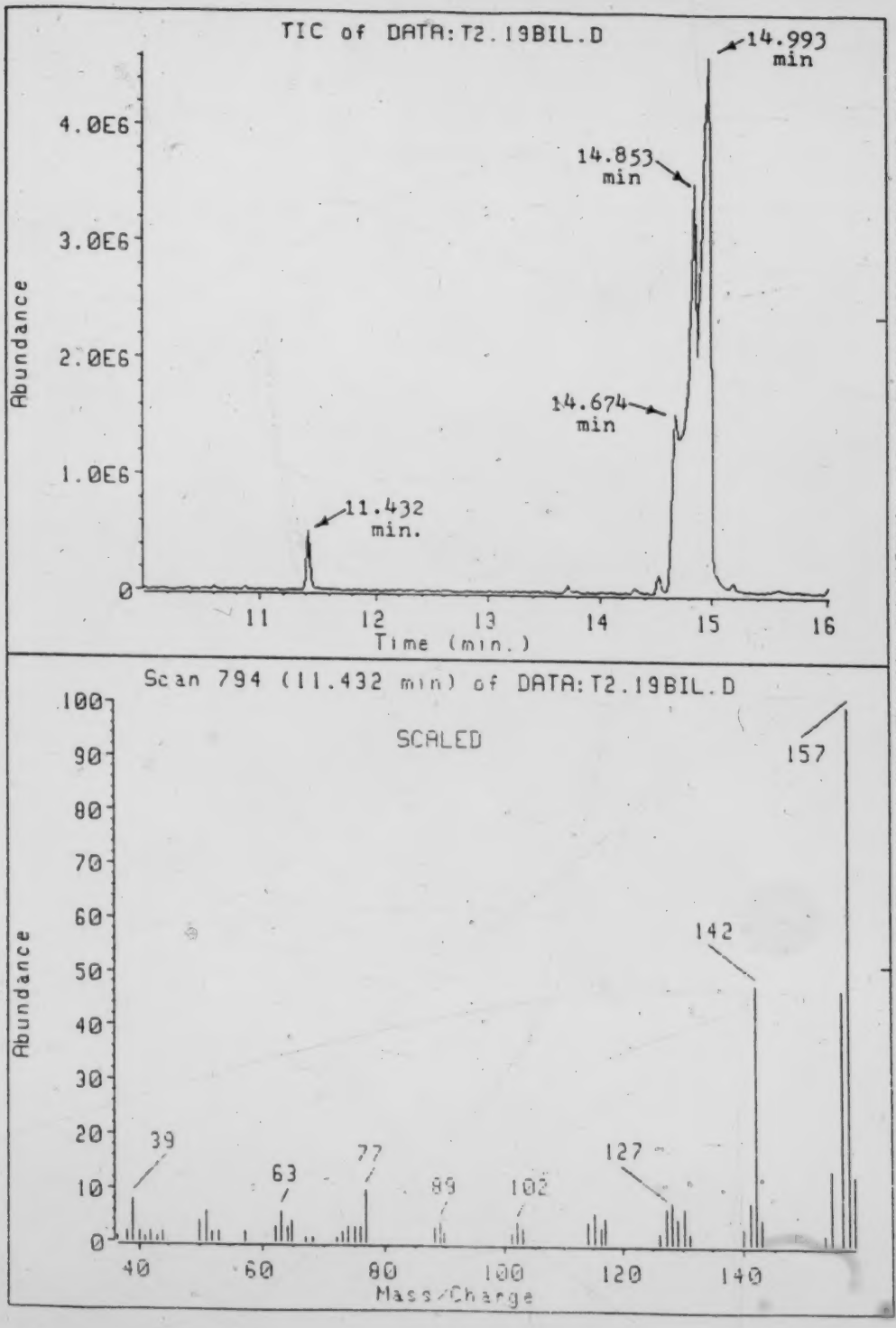

Figure 4-9 TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6,8-DIMETHYLQUINOLINE; LOWER: MASS SPECTRUM FOR GC PEAK APPEARING AT RETENTION TIME 11.432 MIN.

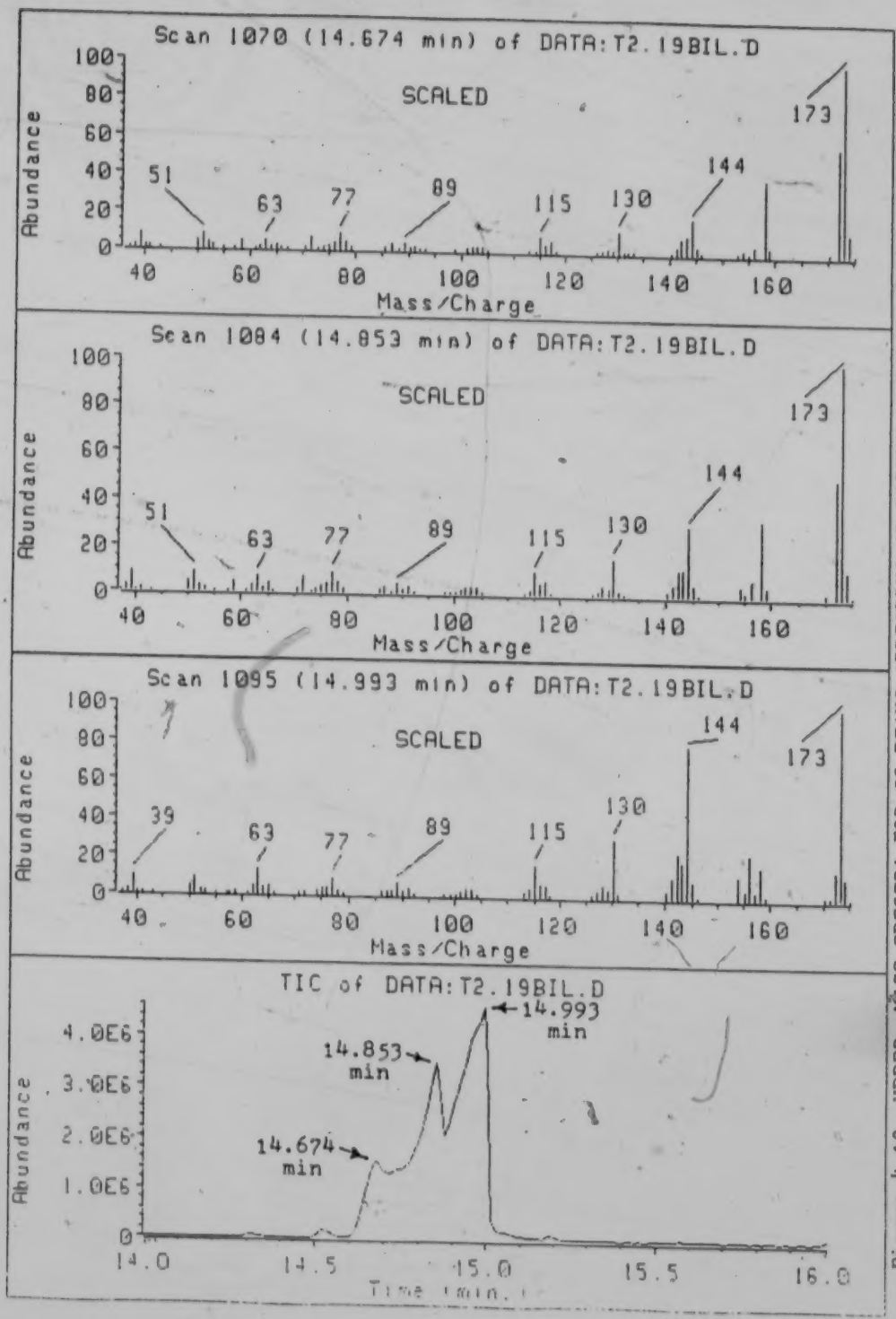

Figure 4-10 UPPER: MASS SPECTRA FOR GC PEAKS APPEARING AT RETENTION TIMES 14.674, 14.853 AND 14.993 MIN.; LOWER: TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6,8-DIMETHYLQUINOLINE

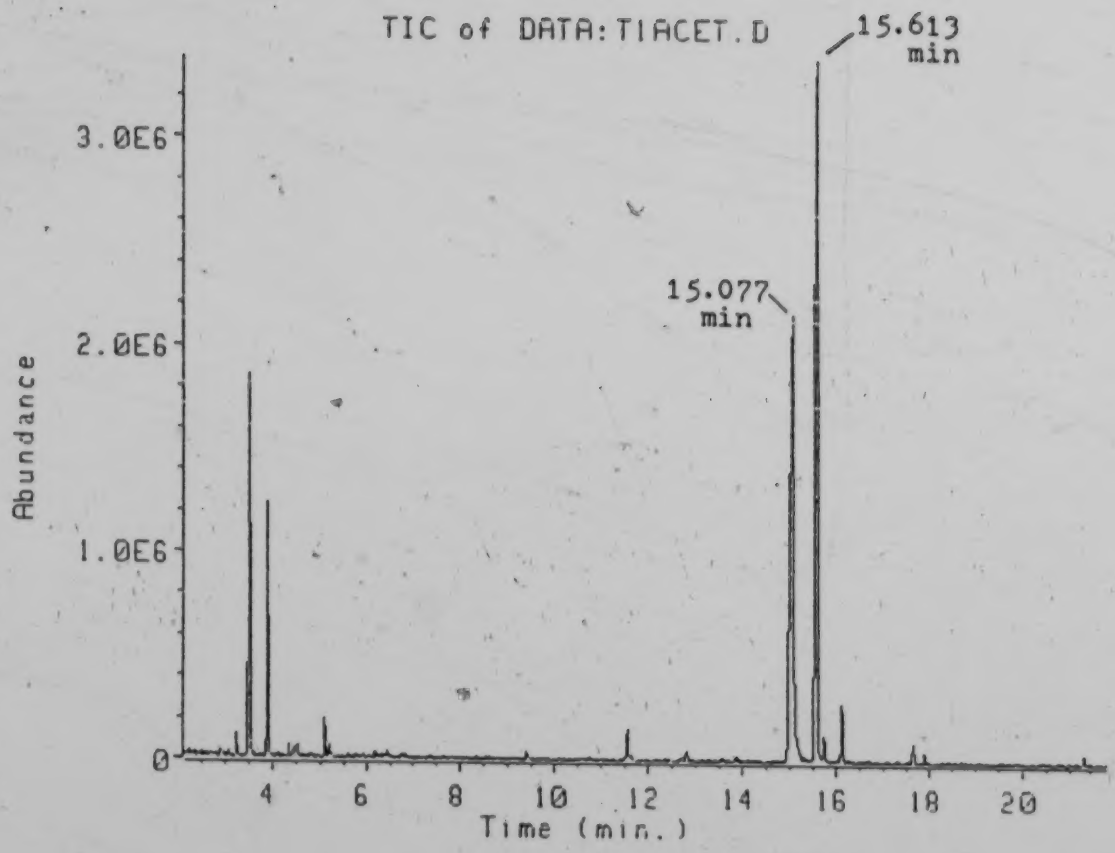

Figure 4-11 TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED, EXTRACTED, AND ACETYLATED BILE OBTAINED FROM FISH EXPOSED TO 6,8-DIMETHYLQUINOLINE

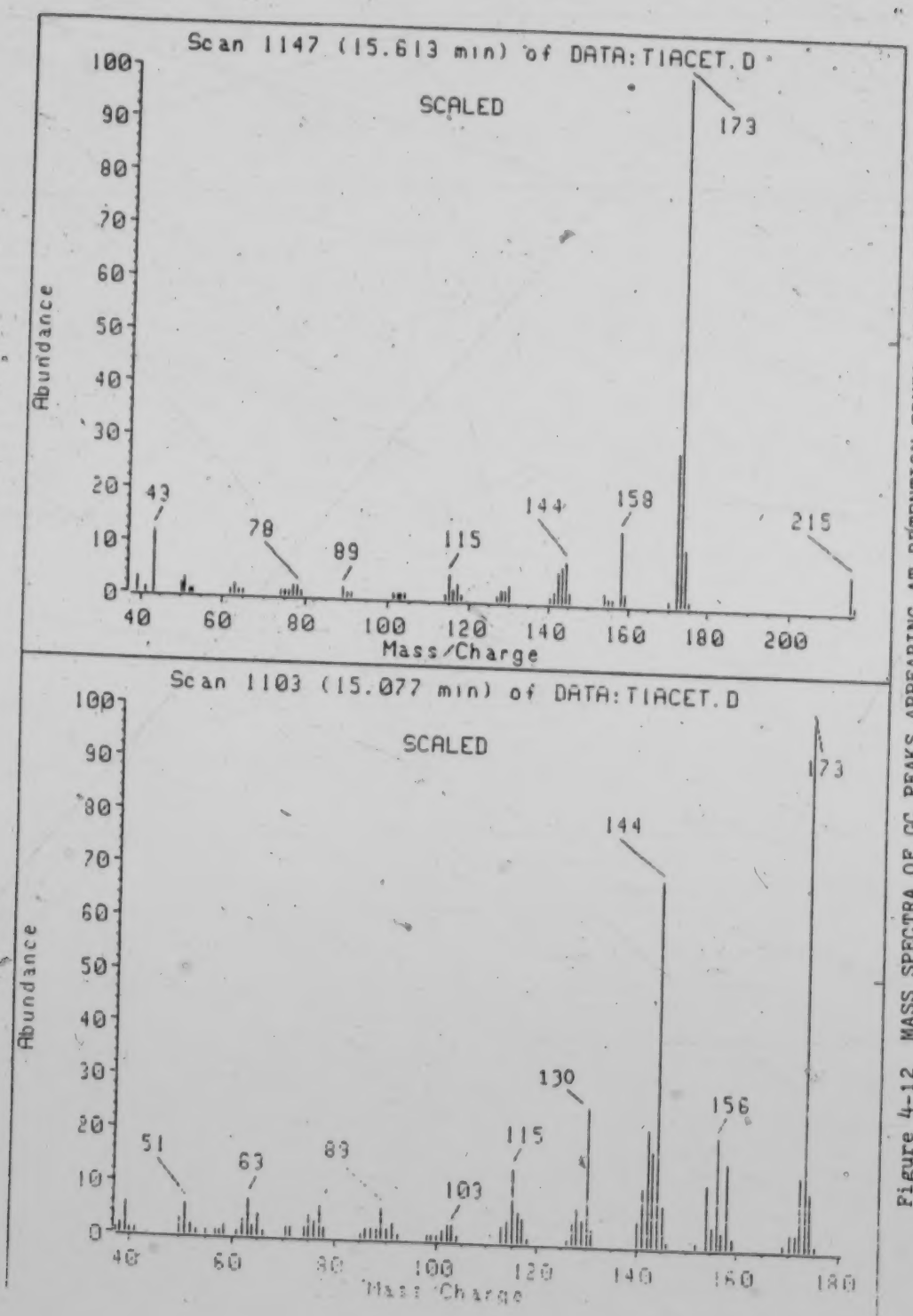

Figure 4-12 MASS SPECTRA OF GC PEAKS APPEARING AT RETENTION TIMES 15.077 AND 15.613 MIN

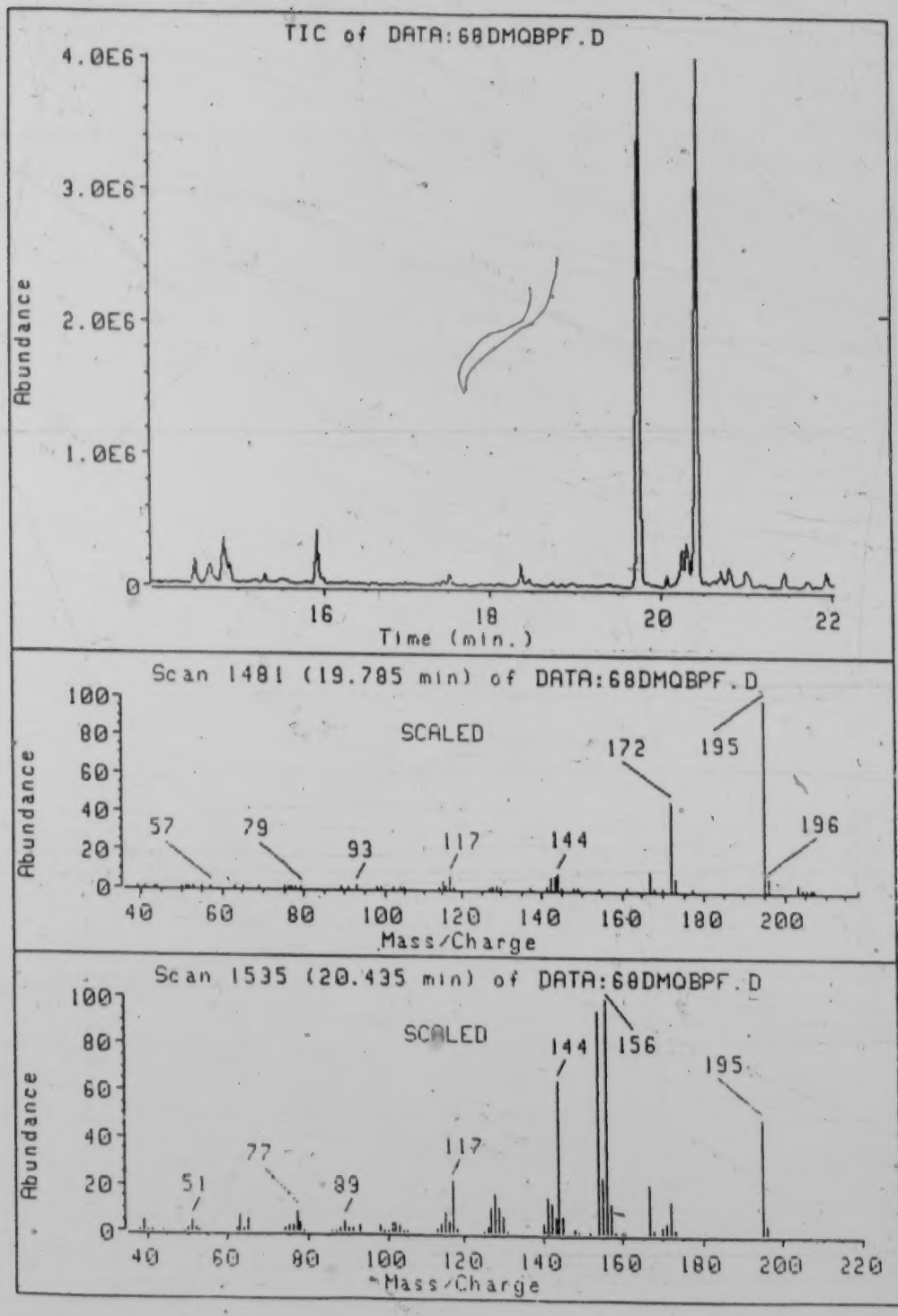

Figure 4-13 UPPER: TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED, EXTRACTED, AND ACYLATED (WITH PFBC) BILE OBTAINED FROM FISH EXPOSED TO 6,8-DIMETHYLQUINOLINE LOWER: MASS SPECTRA FOR GC PEAKS APPEARING AT RETENTION TIMES 19.785 AND 20.435 MIN

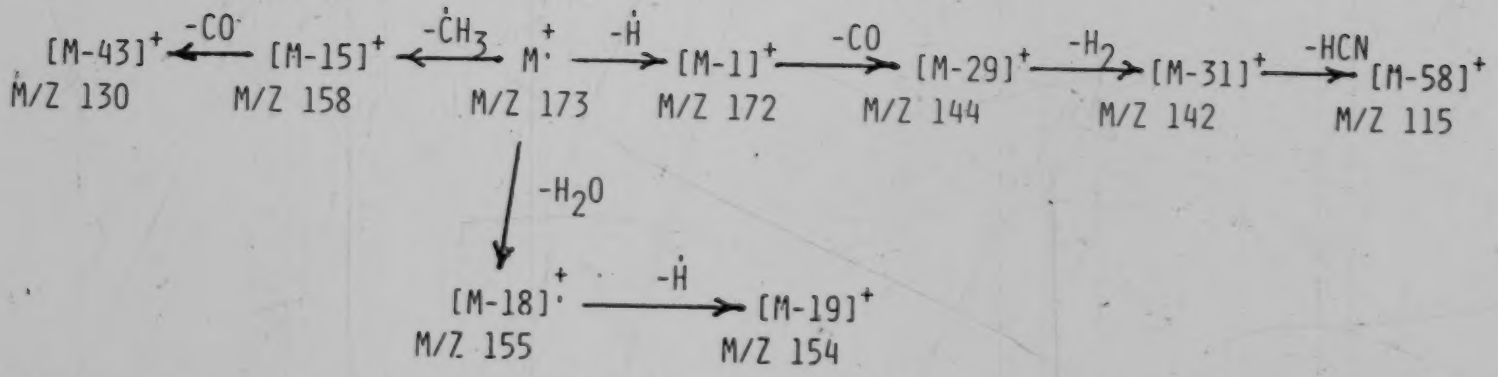

Figure 4-14 POSTULATED FRAGMENTATION MECHANISM FOR MASS SPECTA OF GC PEAKS APPEARING AT RETENTION TIMES 15.588 AND 15.646 MIN. (FIGURE 4-7)

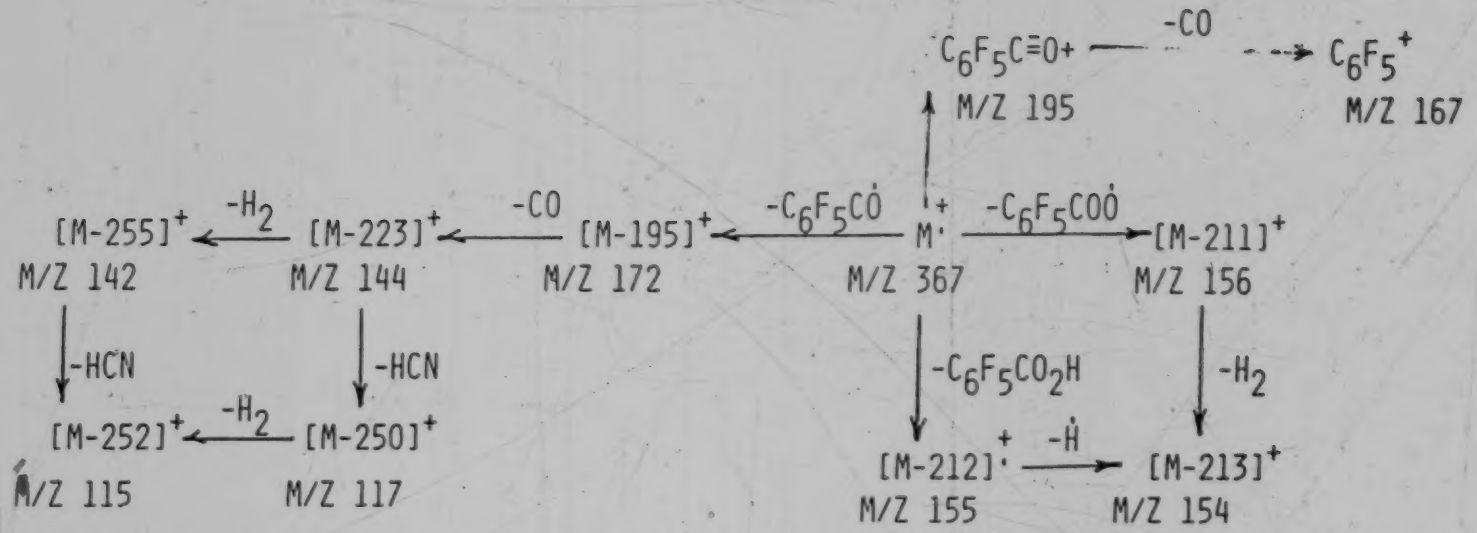

Figure 4-15 MASS SPECTRAL FRAGMENTATION MECHANISM FOR MASS SPECTRA APPEARING AT RETENTION TIMES 20.835 AND 20.976 MIN. (FIGURE 4-8)

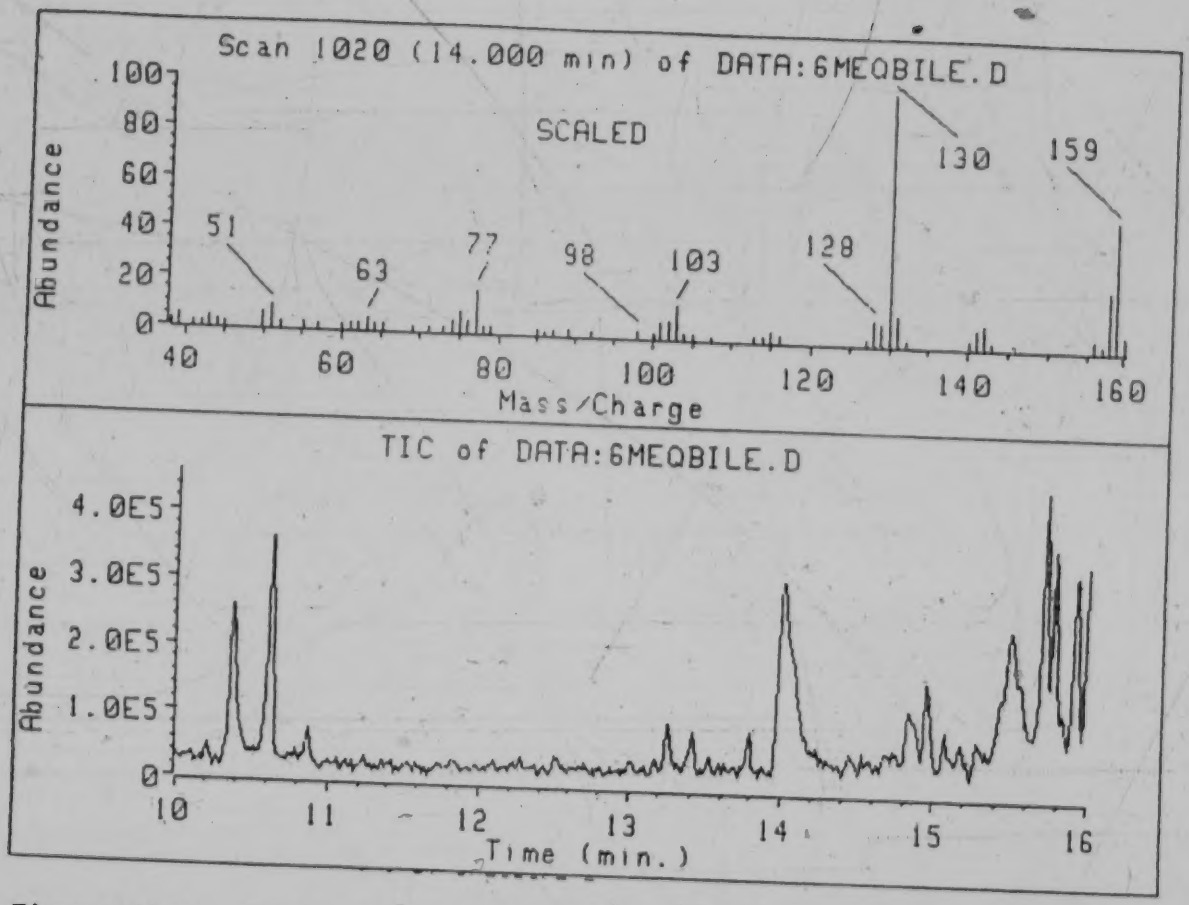

Figure 4-16 UPPER: MASS SPECTRUM OF GC PEAK APPEARING AT RETENTION TIME 14.000 MIN. LOWER: TOTAL ION CHROMATOGRAM OF ENZYMATICALLY HYDROLYZED AND EXTRACTED BILE OBTAINED FROM FISH EXPOSED TO 6-METHYLQUINOLINE

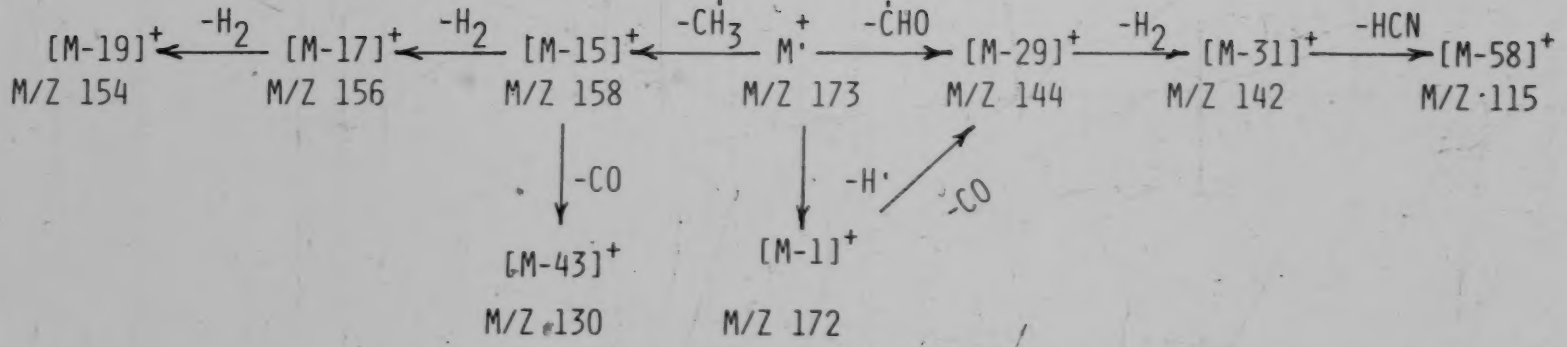

Figure 4-17 POSTULATED FRAGMENTATION MECHANISM FOR 6-HYDROXYMETHYL-8-METHYLQUINOLINE

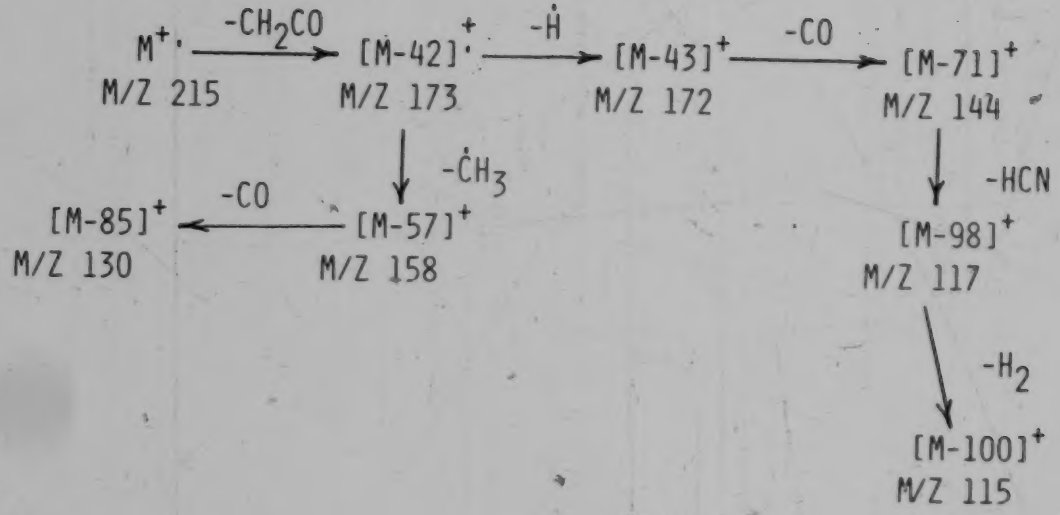

Figure 4-18 POSTULATED FRAGMENTATION MECHANISM FOR ACETYLATED METABOLITE APPEARING AT RETENTION TIME 15.613 MIN (FIGURE 4-12)

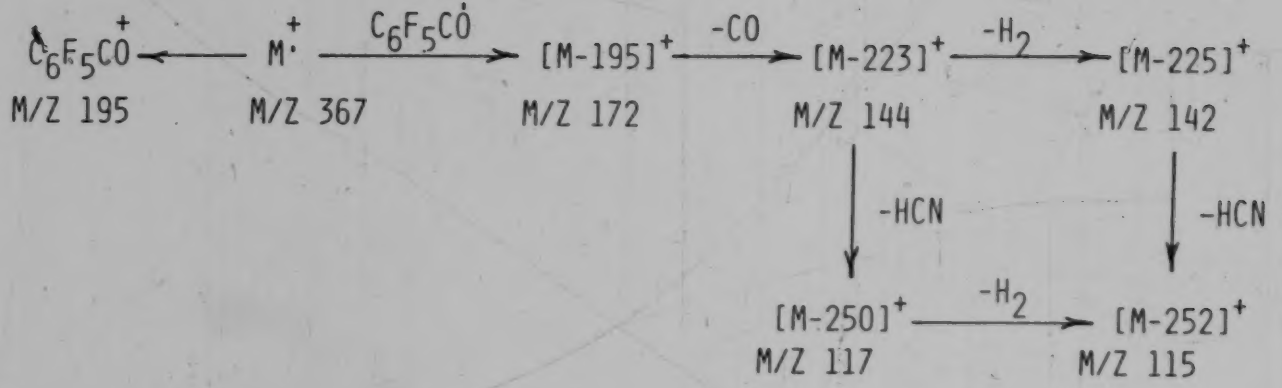

Figure 4-19 PROPOSED FRAGMENTATION MECHANISM FOR PFBC DERIVATIVE OF METABOLITE APPEARING AT RETENTION TIME 19.785 MIN. (FIGURE 4-13).

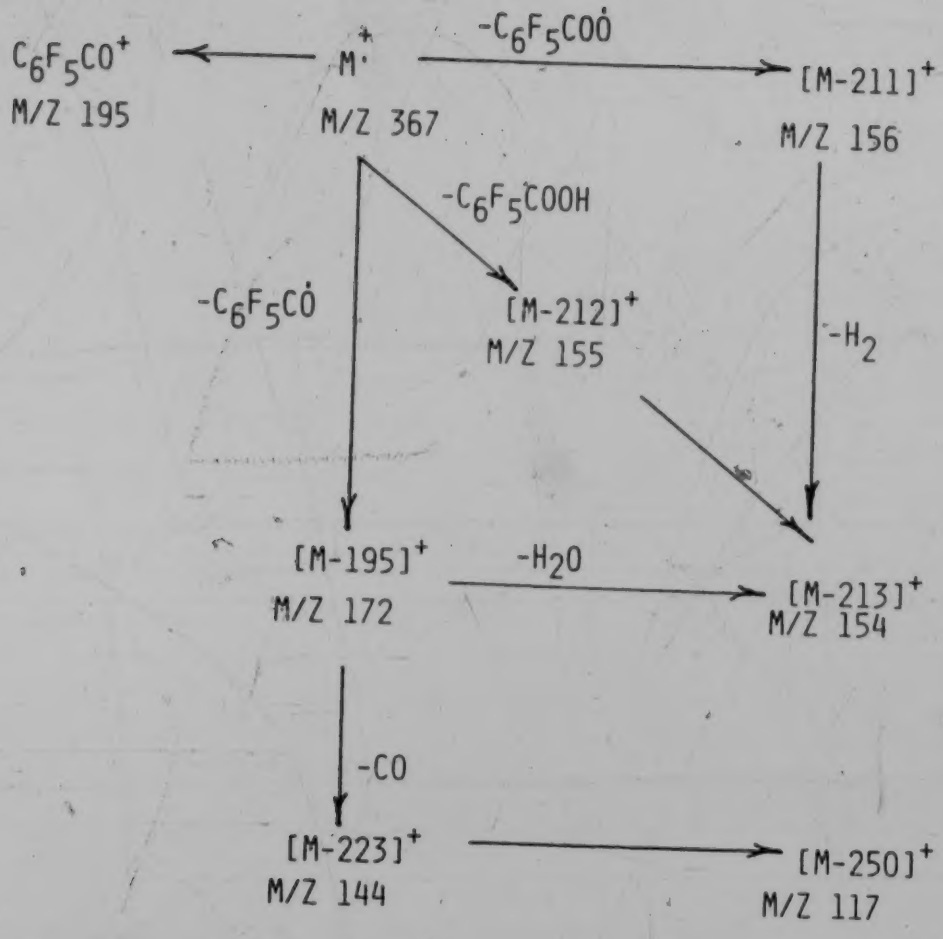

Figure 4-20 PROPOSED FRAGMENTATION MECHANISM FOR PFBC DERIVATIVE OF METABOLITE APPEARING AT RETENTION TIME 20.435 MIN. (FIGURE 4-13)

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5. COMPARATIVE AQUATIC TOXICOLOGY OF ALKYL-QUINOLINES

5.1 Introduction

The occurrence of polycyclic aromatic nitrogen heterocycles (PANH) in various fuels and products of combustion is well-established. Their occurrence in coal tar has been known since the early 1800s (Acheson, 1967); more recently they have been identified in both natural and synthetic crudes (Ford *et al.*, 1981; Tomkins and Ho, 1982; and Schmitter *et al.*, 1982) and in subsequently derived distillates and oils (McKay *et al.*, 1976; Novotony *et al.*, 1980 and Later *et al.*, 1981).

Through the use of fossil fuels PANH have become widespread in the environment (Furlong and Carpenter, 1982; and Wakeham, 1979). Compounds such as quinolines and benzoquinolines have been identified on air particulate matter from Europe (Brocco *et al.*, 1973), the United States (Dong *et al.*, 1977), and the southern North Atlantic Ocean (Hahn, 1980). Recent data indicates that these compounds are also present in ambient air in the vapor phase and at higher levels than previously reported ~~on~~ particulate matter (Adams *et al.*, 1982). PANH have also been found in lake and marine sediments (Furlong and Carpenter, 1982; Wakeham, 1979; and Blumer *et al.*, 1977) and groundwater adjacent to an underground coal gasification site (Stuermer *et al.*, 1982).

While earlier studies pointed out that certain PANH can be more potent carcinogens than the most active polyaromatic hydrocarbons (Lacassagne *et al.*, 1956), recent results of biological testing with fossil fuels (Guerin *et al.*, 1978) reinforce their importance. Many of

these compounds have been reported to be toxic (Sidhu and Blair, 1975 and Schultz *et al.*, 1982), teratogenic (Dumont *et al.*, 1979), mutagenic, and/or carcinogenic (Ho *et al.*, 1979; Hirao *et al.*, 1976; and Dipple, 1976). For example, quinoline and all of its monomethyl isomers were found to be mutagens in the Ames *Salmonella* assay (Dong *et al.*, 1978). Quinoline, 4-methylquinoline, and 8-methylquinoline have also been shown to intiate skin tumors in SENCAR mice (LaVoie *et al.*, 1984).

Considering the many toxic, mutagenic, teratogenic and carcinogenic effects that have been demonstrated for PANH, the widespread distribution of these compounds in the environment should cause concern. More information on the origin, concentration, fate, structure-activity relationships and effects of PANH on the environment is required to assess immediate and long term environmental impacts.

We report on the aquatic toxicity of 21 alkyl-quinolines using luminescent bacteria (*Photobacterium phosphorium*) and on the aquatic toxicity of 3 isomers of dimethylquinoline using rainbow trout (*Salmo gairdneri*). The general structure of quinoline is illustrated in figure 5-1.

5.2. Experimental

5.2.1 Chemicals

Isoquinoline, 6-methylquinoline, 8-methylquinoline, 2,6-dimethylquinoline and 2,4-dimethylquinoline were obtained from Aldrich. The remainder of the substituted quinolines were synthesized in the University of Alberta Chemistry Department using the procedure of Manske *et al.* (1942). Most of the latter were available in limited quantity.

Stock solutions of the substituted quinolines were prepared by weighing the individual compounds into a 10 mL volumetric flask and diluting to volume with dichloromethane. This resulted in a concentration range of 1.5 to 15 mg/mL. Purity of the compounds was determined by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS) to be greater than 97% for all compounds except 7,8-dimethylquinoline and 3,8-dimethylquinoline which were observed to be 69 and 94% pure respectively.

Aqueous solutions of substituted quinolines were prepared by adding aliquots (250 - 1000 μL) of the stock solutions to a 100 mL volumetic flask, removing the solvent by nitrogen evaporation and diluting to volume with distilled and deionized water. Concentrations of the substituted quinolines were determined by high performance liquid chromatography. Other sets of aqueous solutions were prepared similarly for 3,6-, 5,6- and 3,5-dimethylquinoline except that phosphate buffer (pH 7.0) was used as the diluent. The buffer was prepared by titrating 50 mL of 0.1 M potassium dihydrogen

orthophosphate (Fisher Scientific) with 0.1 M sodium hydroxide (Fisher) to pH 7.0.

Ammonium phosphate buffer was prepared by adding 1.0 mL of phosphoric acid (85%) to 1 L of organic free water and titrating to pH 6.5 by dropwise addition of ammonium hydroxide (28 - 30%).

5.2.2 Gas Chromatography and Mass Spectrometry

GC/FID analyses were performed using a Hewlett-Packard (HP), model 5880 gas chromatograph. After injection of 2 μL of diluted stock solution onto a 30 m x 0.32 mm i.d. fused silica, wall-coated DB-1301 capillary column (0.25 μm film thickness, J&W Scientific), the oven temperature was increased from 40 to 280^{o}C at 10^{o}C/min beginning 1 min after injection. The oven temperature was maintained at 280^{o}C for 20 min and the injector and detector were maintained at 270 and 300^{o}C respectively. The FID signal was plotted and integrated using a level four integrator. The carrier gas, helium, maintained a linear velocity of 31 cm/sec at 280^{o}C.

GC/MS analyses were performed on an integrated HP model 5890A GC, an HP model 5970 quadrupole mass spectrometer and a HP model 59970C data system. After injection of 2 μL of diluted stock solution onto a 12.5 m x 0.2 mm i.d. fused silica, wall-coated HP-1 capillary column (0.17 μm film thickness, Hewlett-Packard) the oven temperature was increased from 40 to 300^{o}C at 10^{o}C/min beginning 1 min after injection. The oven temperature was maintained at 300^{o}C for 8 min, and the injector, transfer line and ion source were maintained at 250, 300

and 220°C respectively. Scanning (from 35 to 350 amu) was initiated 2 min after injection at a rate of 1.36 s/scan. The carrier gas, helium, maintained a linear velocity of 35.5 cm/sec at 300°C.

5.2.3. High Performance Liquid Chromatography

High performance liquid chromatography was performed using a Waters (Millipore Corp., Milford, MA) high performance liquid chromatograph. The system consisted of two M6000 pumps, a model 680 solvent programmer, a model 710B autosampler, and a model 450 variable wavelength detector. Separations were performed on a 15 cm x 4.6 mm i.d. Supelcosil LC-PAH, 5-μm column (Supelco, Inc.). Analysis was performed isocratically using acetonitrile (70%) and ammonium phosphate buffer (pH 6.5) as the mobile phase. Flow rate was maintained at 3.0 mL/min and absorbance was monitored at 233 nm. Following injection of 15 to 20 μL of the aqueous substituted-quinoline solutions, chromatograms were recorded and integrated using a Hewlett-Packard model 3388A data system.

5.2.4. Microtox Assay

Bacterial toxicity assays were performed using a Microtox toxicity analyzer. The analyzer, lyophilized luminescent bacteria (*Photobacterium phosphorium*), and other assay reagents were supplied by Microbics Operations of Beckman Instruments, Inc., Carlsbad, Calif.

The instrument was equipped with a photomultiplier tube located near a reaction chamber which had an adjustable temperature range of 10 to 25°C. The analyzer was also equipped with a ten-well incubator chamber which held the test temperature at 15 ± 0.3°C in all assays. Light output was monitored using a chart recorder.

All Microtox bioassays were performed in duplicate following operating instruction and procedures previously described (Bulich *et al.*, 1981 and Lebsack *et al.*, 1981) and are summarized as follows. For all tests 1.8 mL of the aqueous substituted-quinoline solutions and 0.2 mL of 20 percent sodium chloride solution were mixed to yield an initial concentration of 90 percent. All subsequent sample dilutions were made from this solution, using the Microtox diluent, so that 45, 22.5, 11.25 and 5.63 percent of the original concentration were tested.

The Microtox reagent (lyophilized bacteria) was reconstituted with Microtox reconstitution solution, and 0.01-mL aliquots were transferred to cuvettes containing 0.5 mL Microtox diluent equilibrated (about 10 min) at 15°C. Initial light measurements were made three times for each cuvette containing bacterial cell suspension. Diluent control and sample dilutions, equilibrated to 15°C in the incubator wells, were added (0.5 mL) from the corresponding cuvettes to the luminescent bacterial suspensions. Light measurements were then made by cycling cuvettes through the turret, at 1-min intervals, for 5 to 15 min to obtain the final light output readings for each cuvette. The diluent control (blank) was used to correct time-dependent drift in sample light output. The EC_{50} for all

bioassays were calculated using the initial, 5 min and 15 min light levels following the described method of the manufacturer and Bulich (1982). Data reduction employed the gamma function (Γ) which is the ratio of the amount of light lost to the amount of light remaining. Details of this data reduction process are described by Bulich (1982) The EC_{50} values were expressed in milligrams per litre for the substituted-quinolines.

5.2.5. Fish Bioassays

Unaerated 48-h static bioassays were performed on the following chemicals, 6,7-, 6,8-, and 2,6-dimethylquinoline. Young rainbow trout (*Salmo gairdneri*) which had only recently completed resorption of yolk material, and weighed from 80 to 155 mg were used in all bioassays. Exposures were carried out in 4.1 L glass jars which were filled to capacity with dechlorinated Winnipeg city water. Test solutions of the three dimethylquinolines were prepared by dissolving weighed amounts of the chemical in 2 mL of 1,4-diethylene dioxide (1,4-dioxane).

After the addition of fish and the test material, jars were covered with aluminum foil and sealed tightly with screw caps, to prevent volatilization of test material. Only 5 fish were used per jar, and this loading density was low enough that the dissolved oxygen level remained above 60% of saturation, over the 48-h test period. The fish were not fed for 24 h prior to exposure. All exposures were conducted in a controlled environment room with the temperature maintained at 10°C.

Bioassays were also performed on the carrier solvent 1,4-dioxane.

Test concentrations were made by adding 1, 2, 4, and 8 mL of solvent to the 4.1 L jars. These exposures produced no observed effect on the test fish. The maximum trial exposure was 1.9 mL/L compared with less than 0.4 mL/L in the quinoline bioassays. Test solutions were obtained by adding 2 mL solvent to the vial containing the dimethylquinoline. The dissolved extract was then poured into a flask containing 9 L of rapidly stirred test water and a second 2 mL was also added from the vial rinse. This 9 L of stock solution was then diluted with differing amounts of solvent-fortified water (4 ml of 1,4-dioxane per 9 L of water) to obtain the test concentrations.

5.2.6. Quality Control and Quality Assurance

Quality control/quality assurance was maintained by repeated testing of an aqueous solution (ca. 10 mg/L) of hexachloroethane (n=16) during all Microtox testing of substituted quinolines.

5.3 Results

Quality control data for the Microtox assay revealed reasonable precision and adequate agreement with published values for hexachloroethane. Our mean 5-min EC_{50} (5-EC_{50}) was 0.31 mg/L (n=16, s.d.=0.08 mg/L) compares with the value of 0.14 mg/L reported by Curtis *et al.* (1982). The method precision, reflected by a relative standard deviation (RSD) of 25% over the course of the testing program, provides a reasonable basis for comparison of toxicity values.

All aqueous solutions of substituted quinolines were made up in slightly acidic water (pH 5.1). To check whether any dissociation of the quinolines, which are strong bases, was occurring, solutions of 3,6-, 5,6-, and 3,5-dimethylquinoline were prepared in pH 7 buffer and retested. No significant differences were observed for either 5-min or 15-min EC_{50} values compared with test solutions prepared at pH 5.1. For example 5-EC_{50} values determined for 3,6-, 5,6-, and 3,5-dimethylquinoline at pH 5.1 were 0.30, 0.74 and 1.0 mg/L respectively. Similar values were obtained for 3,6-, 5,6-, and 3,5-dimethylquinoline at pH 7, namely, 0.36, 0.78 and 1.2 mg/L respectively. Fifteen-min EC_{50} (15-EC_{50}) obtained for these chemicals at pH 7 were observed to be within a factor of 1.4 of those obtained at pH 5.1.

A summary of the Microtox test results observed for the 21 quinoline compounds is provided in table 5-1. All compounds demonstrated linear responses for log gamma vs log of concentration thereby providing a sound basis for calculating the EC_{50}. Only small differences were measured between 5-min and 15-min values but very large differences are apparent among compounds. For example, toxicity of the dimethylquinolines varies over two orders of magnitude from 0.30 mg/L (5-EC_{50}) for 3,6-dimethylquinoline to 29.9 mg/L (5-EC_{50}) for 2,4-dimethylquinoline. Toxicity for the related compounds, isoquinoline, isomers of methylquinoline, 3-ethylquinoline, 3-isopropylquinoline, and 2-methyl-8-ethylquinoline fell within the wide range exhibited by the isomers of dimethylquinoline.

Median lethal toxicity values for rainbow trout (48h-LC_{50}) are reported in table 5-2 for three isomers of dimethylquinoline which

were available in sufficient quantity. Unfortunately, the synthesized dimethylquinolines which exhibited extreme values in the Microtox test were not available in sufficient quantity to perform fish bioassays.

5.4. Discussion

The wide range of toxicities observed for the isomers of dimethylquinoline is notable, particularly compared with published toxicity ranges for substituted aromatic compounds. Saethre *et al.* (1984) found tolerance limits ranging from 0.6 to 1.8 mg/L for newly fertilized sea urchin and cod eggs when exposed to naphthalene and its mono- and dimethylated derivatives. Ribo and Kaiser (1983) reported 5-EC_{50} for dichlorophenols varied from 3.9 to 8.3 mg/L and 5-EC_{50} for dichlorobenzenes varied from 2.7 to 4.3 mg/L. Liu *et al.* (1982) found an IC_{50} using the test bacterium *Bacillus sp.* (TL81) for dichlorophenols to vary over a wide range from 25 to 550 mg/L. The term IC_{50} used in their study refers to the effective concentration of the toxicant causing 50% inhibition of the bacterial dehydrogenase activity.

The observed differences in toxicity raise the question of what factors may be responsible. A variety of possible explanations have been applied to explain toxicity differences among closely related compounds.

5.4.1. Octanol-Water Partition Coefficient

Good correlation between observed toxicity of various chemicals and the octanol-water partition coefficient and/or water solubility

have been reported in several cases. Such correlations have been reported for toxicity testing with algae (Hutchinson *et al.*, 1980), Microtox (Ribo and Kaiser, 1983), fermentative and oxidative yeasts (Kwasniewska and Kaiser, 1983), bacteria (Liu *et al.*, 1982), rainbow trout (*Salmo gairdneri*) (Black *et al.*, 1983) and fathead minnow (*Pimephales promelas*) (Veith *et al.*, 1983).

Ribo and Kaiser (1983) reported a correlation between the logarithm of octanol-water partition coefficient (log P) and the 30 min EC_{50} for chlorophenols and chlorobenzenes. A plot of log (1/C) versus log P gave a positive correlation for both chlorophenols (r^2=0.79, n=20) and chlorobenzenes (r^2=0.76, n=11). C is expressed as the 30-EC_{50} in mmol/L.

Published octanol-water partition coefficients were available for isoquinoline, 6-methylquinoline, 8-methylquinoline and 2,6-dimethylquinoline (Leo *et al.*, 1971). Using these data, the correlation coefficient, r, for log (1/C) vs log P was only -0.25, indicating a lack of significant correlation.

Another approach, to include the data for those compounds without log P values, was pursued. A good correlation has been reported between retention time measured by reverse phase liquid chromatography, using water/acetonitrile as the mobile phase, and the octanol-water partition coefficient (Swann *et al.*, 1983 and Wasik *et al.*, 1983). Swann *et al.* (1983) provided the equation:

$$\text{Log P} = 3.446 \text{ Log RT} + 1.029 \qquad (1)$$

and reported agreement between calculated and measured values approximately within a factor of 2. The applicability of this range to

our data was tested by calculating the log P values and comparing them with the values provided by Leo *et al.* (1971) for isoquinoline, 6-methylquinoline, 8-methylquinoline and 2,6-dimethylquinoline. The largest disagreement observed was for 8-methylquinoline which gave a calculated log P value of 1.13, compared with the published value of 2.6

Based upon the logic of equation 1, log (1/C) was tested for correlation with the log of liquid chromatography retention time (log RT). This evaluation produced a correlation coefficient of only 0.14 (n=21). Consequently, there was no evidence that octanol-water partition coefficient could substantially explain the observed differences in toxicity among the quinoline compounds.

5.4.2. Molecular Volume

McGowan and Mellors (1986) have proposed a correlation between the molecular volume (V_X, in m^3 mol^{-1}) of a chemical and its toxic concentration in water (C, in mol m^{-3}). They presented the relationship:

$$\text{Log } C = -3600\ V_X + 2.1 \qquad (2)$$

This method does not distinguish between isomers, so its application to our results was limited to the cases presented in table 5-3. These data indicate that the predicted toxicity based upon the molecular volume correlation is near the most toxic end of the range of toxicities observed among the isomers of methylquinoline and dimethylquinoline. In the case of isoquinoline (which could not be distinguished from quinoline for the calculation) agreement between

the observed and calculated values was reasonable.

For the cases of 3-ethylquinoline, 3-isopropylquinoline and 2-methyl-8-ethylquinoline, the predicted values are much lower than the measured values. Unfortunately, other isomers of these compounds were not available for testing to determine if some might be in closer agreement with the predictions. Based on the comparison for methylquinolines and dimethylquinolines, the possibility of other isomers providing closer agreement with the calculated values cannot be ruled out.

Conclusions which can be drawn are restricted by the range of compounds which we were able to test. However, the results obtained are consistent with the possibility that the molecular volume correlation predicts a maximum toxicity which may be exerted by a group of isomers. Variations in the substitution pattern might then be responsible for reducing toxicity from this maximum value.

5.4.3. Specific Structure

Correlation of observed toxicity with some basic properties of the chemicals tested was not obtained thereby making some other explanation necessary. Although quinoline compounds have a planar structure, the orientation of substituents might affect the affinity of the chemical to binding sites on specific enzymes. Pursuing that possibility will require an understanding of the specific mode of toxic action of these chemicals. However, some observations concerning the patterns of substitution and their impact on observed toxicity are possible.

Figures 5-2 and 5-3 show the 5-min EC_{50} values displayed at the substitution locations for the mono- and disubstituted positions respectively. Bearing in mind the experimental precision indicated by a relative standard deviation of 25%, some patterns are apparent.

The most notable pattern is that substitution at the 2 position corresponds to lower toxicity. This is evident for the dimethylquinolines with 4, 6, 7 and 8 positions combined with a 2 position. In every case, the combination involving a 2 position shows the lowest toxicity among the range of toxicities for isomers involving the other position. This observation suggests that the proximity of a substitution adjacent to the ring nitrogen reduces the toxic effect. It should be noted that the 2-position of the quinoline nucleus is most electron deficient and therefore most reactive to nucleophiles. Substitution of this position would be expected to reduce toxicity because it would be no longer available to react with tissue nucleophiles. The observed toxicities may therefore be related to electronic effects, i.e. susceptability to nucleophilic attachment.

Other patterns are apparent from comparison of figures 5-2 and 5-3. Dimethylquinolines involving positions 5 or 6 together with a 3 position are more toxic than the quinolines mono-substituted at the 3 position. Likewise, substitution at the 3 or 5 position results in greater toxicity for di-substituted quinolines including a 6 position and substitution at the 3, 4 or 6 position results in greater toxicity for di-substituted quinolines including an 8 position.

5.4.4. Fish Toxicity

The LC_{50} values reported in table 5-2 for rainbow trout indicate a similar level of toxicity as that determined by the Microtox test. These results demonstrate the relevance of the Microtox data as a means for initial screening of chemicals for aquatic toxicity. Unfortunately, no conclusions can be drawn about a general correlation in toxicity between fish bioassays and the Microtox assay for the full range of quinoline compounds. However, the high toxicity observed for some of the dimethylquinolines in the Microtox test suggests that synthesis of these compounds in sufficient quantity to perform fish bioassays may be warranted.

5.5. Summary

Toxicity testing on 21 quinoline derivatives demonstrated that all compounds are moderately toxic according to the Microtox assay. Acute lethal bioassays on rainbow trout for three of these compounds demonstrated a similar level of toxicity.

The range of toxicity observed for dimethylquinoline isomers spans two orders of magnitude. This variability does not correlate with the octanol-water partition coefficient. Calculation of toxicity by relation to molecular volume provides an estimate for the maximum observed toxicity. However, this method does not distinguish isomers and therefore cannot explain the reduced toxicity apparent for many isomers. Explanation of these differences must depend upon factors associated with substitution patterns. The most notable of these is that dimethylquinolines which include a 2-substitution are less toxic than those of isomers without a 2-substitution.

5.6. TABLES

Table 1 Microtox Assay of Substituted Quinolines

Compound	5-min EC_{50} (mg/L)	15-min EC_{50} (mg/L)
3,6-dimethylquinoline	0.30	0.36
5,6-dimethylquinoline	0.74	0.91
5-methylquinoline	0.95	0.98
3,5-dimethylquinoline	1.0	1.1
6,7-dimethylquinoline	1.3	1.9
4,8-dimethylquinoline	1.7	2.0
isoquinoline	1.7	2.2
6,8-dimethylquinoline	2.2	2.4
6-methylquinoline	2.2	2.8
4,6-dimethylquinoline	4.0	4.4
3,7-dimethylquinoline	4.5	3.8
3,8-dimethylquinoline	4.6	5.1
2,6-dimethylquinoline	5.7	6.3
3-ethylquinoline	6.3	7.8
7,8-dimethylquinoline	7.0	7.9
2,7-dimethylquinoline	9.6	11.
8-methylquinoline	8.8	9.1
3-isopropylquinoline	10.	9.5
2-methyl-8-ethylquinoline	14.	13.
2,8-dimethylquinoline	14.	14.
2,4-dimethylquinoline	30.	22

Note: All compounds produced linear plots of log È vs log concentration.

Table 5-2 Median Lethal Concentration of Some Quinoline Derivatives to Rainbow Trout (Salmo gairdneri)

Compound	Rainbow Trout 48-h LC_{50} (mg/L)	Microtox 5 min- EC_{50} (mg/L)
2,6-dimethylquinoline	6.2	5.7
6,7-dimethylquinoline	7.6	1.3
6,8-dimethylquinoline	2.6	2.2

Table 5-3 Comparison of 5-min EC_{50} values as determined with the Microtox test to calculated toxicity values obtained from molecular volume theory

Compound	5-min EC_{50} (mg/L)	Calculated toxicity (mg/L)
dimethylquinolines	0.30 - 30	0.31
methylquinolines	0.95 - 8.8	1.01
isoquinoline	1.7	2.8
3-ethylquinoline	6.3	0.33
3-isopropylquinoline	10.	0.11
2-methyl-8-ethylquinoline	14.	0.11

5.7. FIGURES

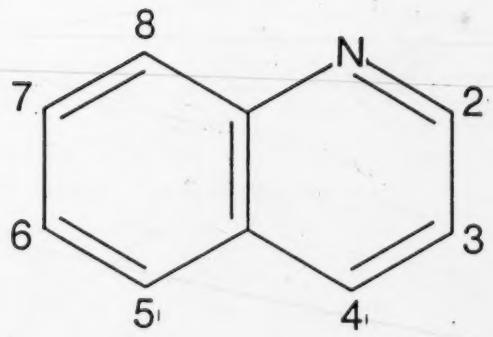


Figure 5-1 BASIC STRUCTURE AND NOMENCLATURE FOR QUINOLINES

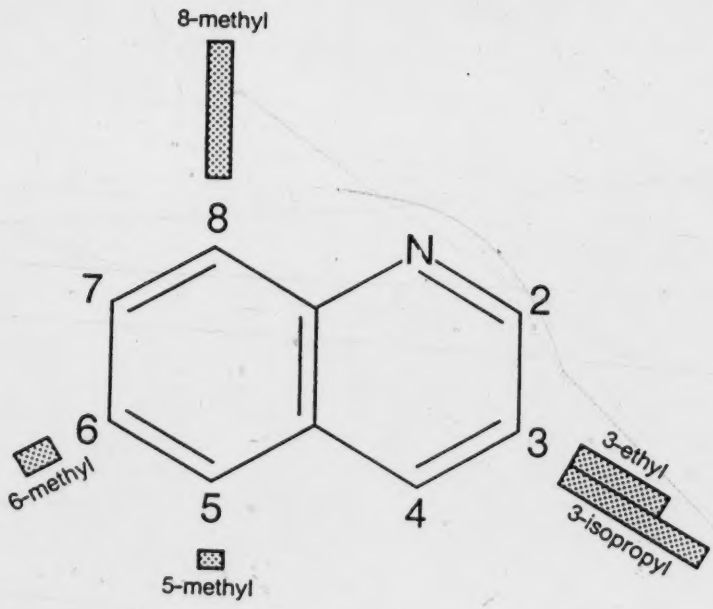
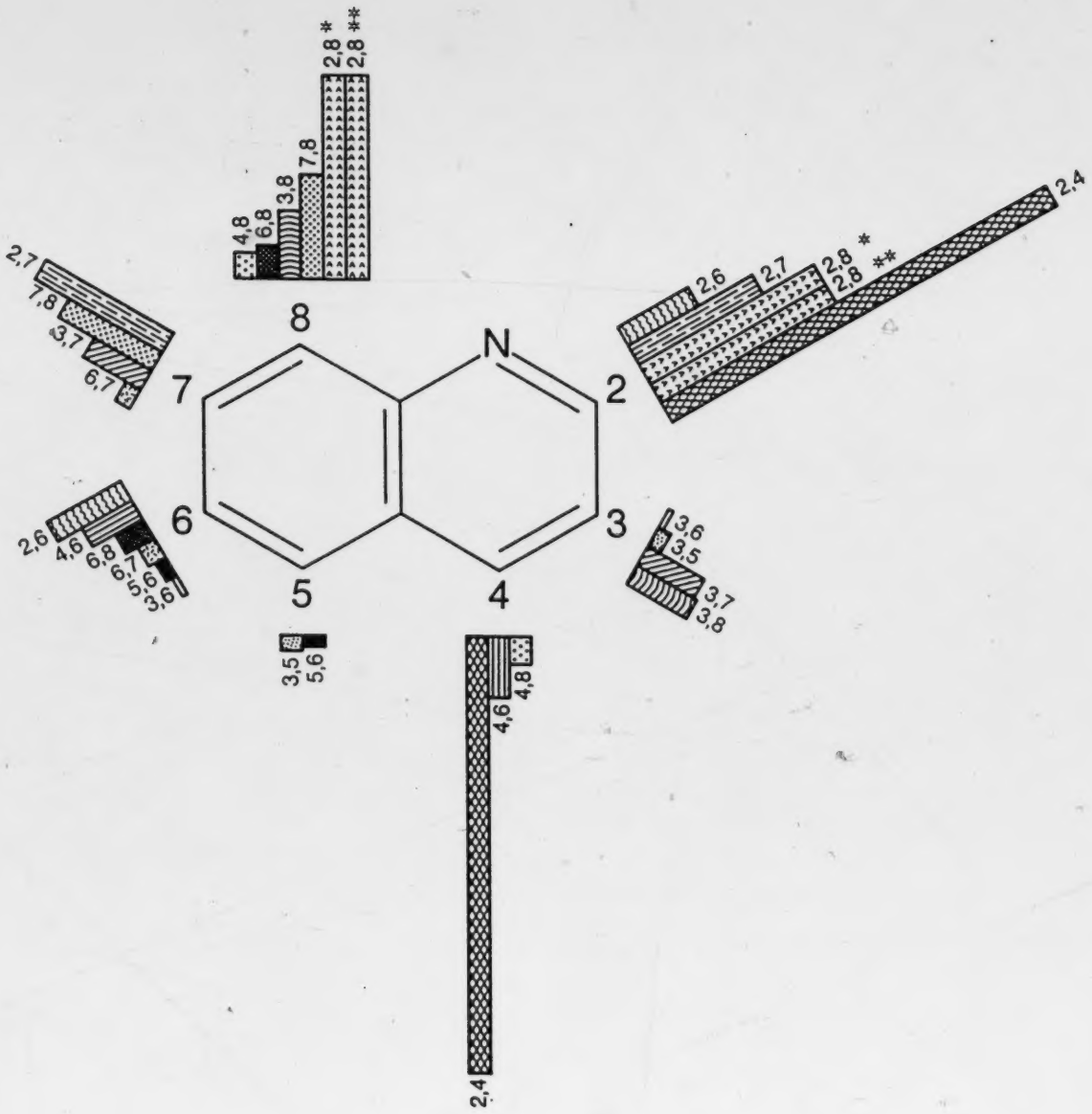


Figure 5-2 TOXICITY OF MONO-SUBSTITURED QUINOLINES; BAR VALUES PROPORTIONAL TO 5-MIN EC_{50}



* 2,8-dimethylquinoline
** 2-methyl-8-ethylquinoline

FIGURE 5-3 TOXICITY OF DI-SUBSTITUTED QUINOLINES; BAR VALUES PROPORTIONAL TO 5-MIN EC_{50}

5.8. References

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6.0 CONCLUSIONS

Samples from two of the major process streams present in a heavy oil surface mining and upgrading plant were subjected to chemical fractionation and bioassay using the Microtox test and the Ames test. The most toxic sample (as determined with the Microtox test) was observed to be coker distillate. Coker distillate is comprised of coker gas oil and coker naphtha (2:1, v/v). The most genotoxic sample (as determined with the Ames test) was observed to be coker distillate. The most toxic chemical fraction isolated from coker distillate (as determined with the Microtox and Ames test) was observed to consist of strongly basic polycyclic aromatic nitrogen heterocycles (basic-PANH). Gas chromatography/high resolution mass spectrometry analysis of this fraction revealed the presence of alkyl-substituted quinolines and alkyl-substituted polycyclic aromatic nitrogen heterocycles.

Following the development of an analytical method for the determination of basic-PANH in fish, experiments were intiated to study the uptake and biotransformation of 6,7-dimethylquinoline and 6,8-dimethylquinoline by fish. Both chemicals were readily bioconcentrated by fish from water and readily eliminated during depuration. However, significant levels of metabolites remained in the bile of exposed fish after 63 h depuration with feeding. These levels were three orders of magnitude greater than the intial exposure levels. The major metabolites of 6,7-dimethylquinoline were observed to be conjugated (sulfate of glucuronide) alcohols, whereas the major metabolites of 6,8-dimethylquinoline were observed to be conjugated

(sulfate or glucuronide) phenols and an alcohol. The significance of these high levels of metabolites present in the bile of exposed fish after depuration is unknown.

The analysis of bile for metabolites may provide an effective tool for environmental monitoring of toxic chemicals such as substituted quinolines. Fish exposed to 6,7- and 6,8-dimethylquinoline concentrated metabolites in the bile at levels far exceeding the concentration of parent compound in the muscle. Although the data presented in this research is limited, a trend toward the bioconcentration of metabolites of substituted quinolines in the bile of exposed fish is evident. The bioconcentration of metabolites in the bile of fish exposed to xenobiotics is presently poorly understood and, although the work presented in this thesis is exploratory, the findings are significant.

Substituted quinolines (21) were subjected to toxicity testing using the Microtox test. Toxicity (expressed as 5-min EC_{50}) was found to vary over two orders of magnitude (from 0.30 to 30 mg/L) depending on the degree and type of chemical substitution. Greatest toxicity was observed for dimethylquinolines substituted at the 3 or 5 position including a 6 position, and least toxicity was observed for quinolines substituted in the 2 position.

Three dimethylquinolines were subjected to static fish bioassay using rainbow trout and the observed 48h-LC_{50} values indicated a similar level of toxicity as that determined by the Microtox test.

In light of the above observations it is concluded that the release of coker-distillate fractions to the environment should be of

environmental concern. Alkyl-substituted quinolines which are present in coker distillate are not only significantly lethal to fish and genotoxic, but are readily bioconcentrated by fish from water. Although these compounds are readily eliminated from fish upon depuration, high levels of metabolites remain in the bile of fish for days after depuration and feeding. Levels of metabolites observed in the bile of fish after depuration and feeding were three orders of magnitude above exposure levels. Since basic-PANH isolated from coker distillate were found to be very genotoxic after liver activation (as determined with the Ames test), these metabolites may affect the physiological health of the fish by promoting neoplasms and other genetic diseases.

Accordingly, regulatory agencies should be concerned about the release of substituted quinolines to the environment and efforts should be expended towards monitoring for the presence of these compounds in receiving streams adjacent to heavy oil development and in fish.